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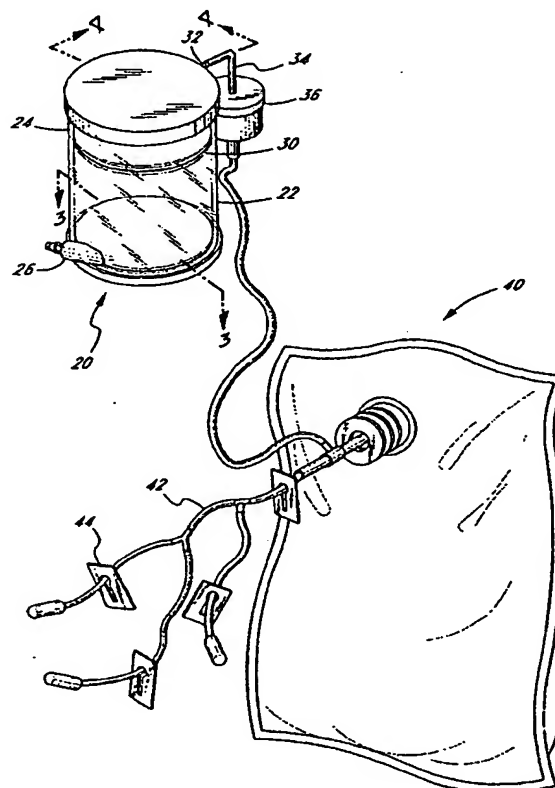
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY

(51) International Patent Classification 5 : <b>B01F 3/12</b>	<b>A1</b>	(11) International Publication Number: <b>WO 94/22566</b> (43) International Publication Date: 13 October 1994 (13.10.94)
<p>(21) International Application Number: PCT/US94/03652</p> <p>(22) International Filing Date: 4 April 1994 (04.04.94)</p> <p>(30) Priority Data: 08/041,915 2 April 1993 (02.04.93) US 08/043,581 6 April 1993 (06.04.93) US</p> <p>(71) Applicant: IRVINE SCIENTIFIC SALES CO. [US/US]; 2511 Daimler Street, Santa Ana, CA 92705-5588 (US).</p> <p>(72) Inventors: WALTHALL, Ben, J.; 10431 Boca Canyon Drive, Santa Ana, CA 92705 (US). MURPHY, Thomas, J.; 4532 Vincent Avenue South, Minneapolis, MN 55410 (US).</p> <p>(74) Agent: ALTMAN, Daniel, E.; Knobbe, Martens, Olson &amp; Bear, Suite 1600, 620 Newport Center Drive, Newport Beach, CA 92660 (US).</p>		<p>(81) Designated States: AU, BR, CA, JP, KR, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p><b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>

(54) Title: DISSOLUTION APPARATUS

## (57) Abstract

Disclosed is a unit volume dissolution apparatus (20) for reconstituting a one or more component concentrated media in an influent stream and is adapted to fit integrally with fluid-receiving receptacle (40). Dissolution is facilitated by a fluid-driven mixing vortex. The effluent fluid stream (32) is filtered (at 36), sterilized and delivered directly into a fluid-receiving receptacle (40). Also disclosed is a unit volume mixing apparatus (20) for reconstituting a one or more component concentrated media in an influent stream (at 26). Mixing is facilitated by a water-driven mixing vortex. The effluent fluid stream (32) is filtered, sterilized and delivered to a sterilized receiving bag (40) for containing a unit volume of reconstituted material.



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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/03652

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) :B01F 3/12

US CL : 366/165, 176, 189, 167, 168

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 366/165, 176, 189, 167, 168, 173, 150, 340, 341; 239/10, 400, 404, 402, 405

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,218,012 (HAMZA ET AL) 19 AUGUST 1980	1-24
A	US, A, 4,511,256 (KARG ET AL) 16 APRIL 1985	1-24
A	US, A, 4,726,686 (WOLF ET AL) 23 FEBRUARY 1988	1-24
A	US, A, 4,729,663 (KARG) 08 MARCH 1988	1-24
A	US, A, 4,795,092 (FULLER) 03 JANUARY 1989	1-24
A	US, A, 4,398,827 (DIETRICH) 16 AUGUST 1983	1-24
A	US, A, 4,415,275 (DIETRICH) 15 NOVEMBER 1983	1-24

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search  
11 JULY 1994

Date of mailing of the international search report  
29 JUL 1994

Name and mailing address of the ISA/US  
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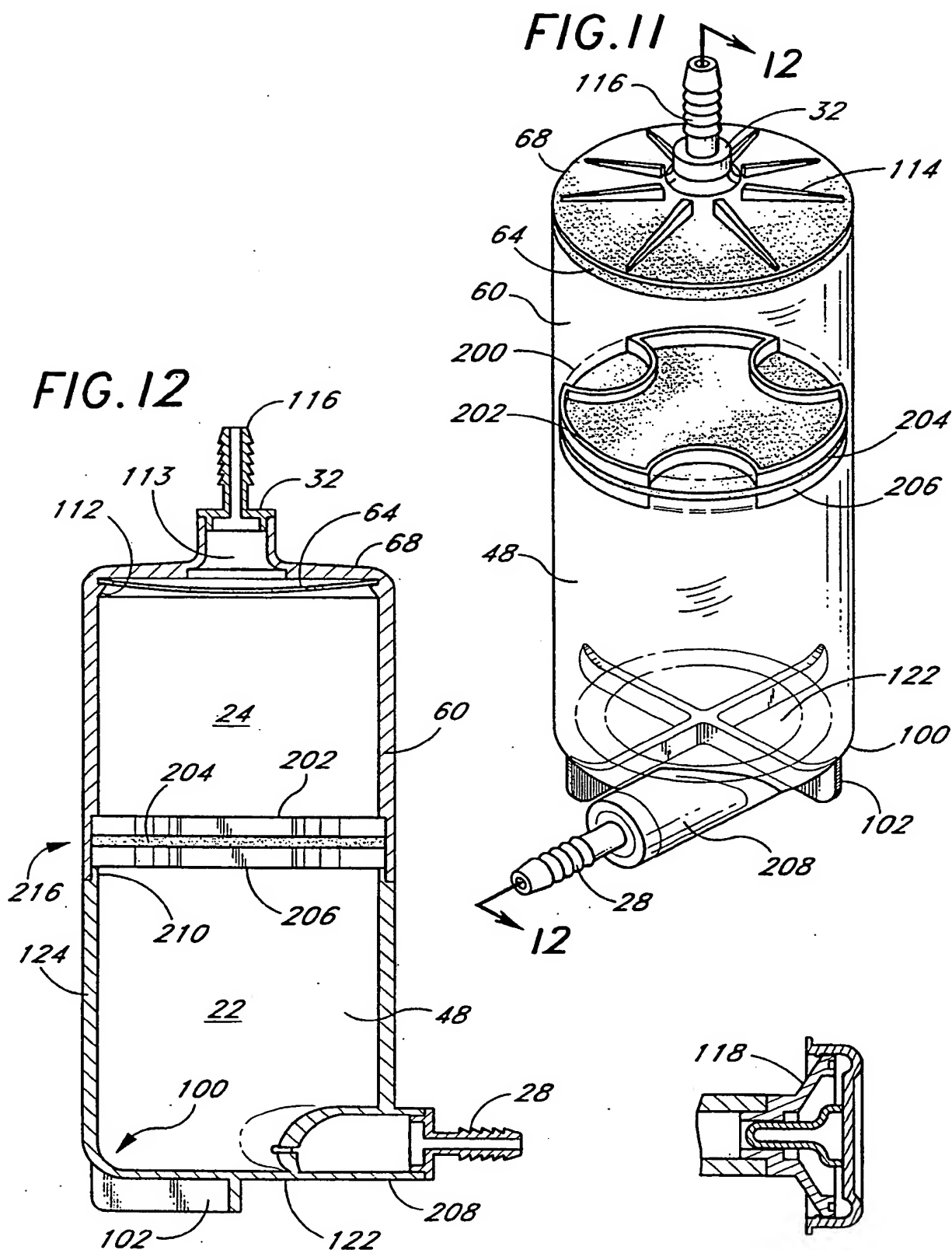
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**FIG. 13**

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**FIG. 10**

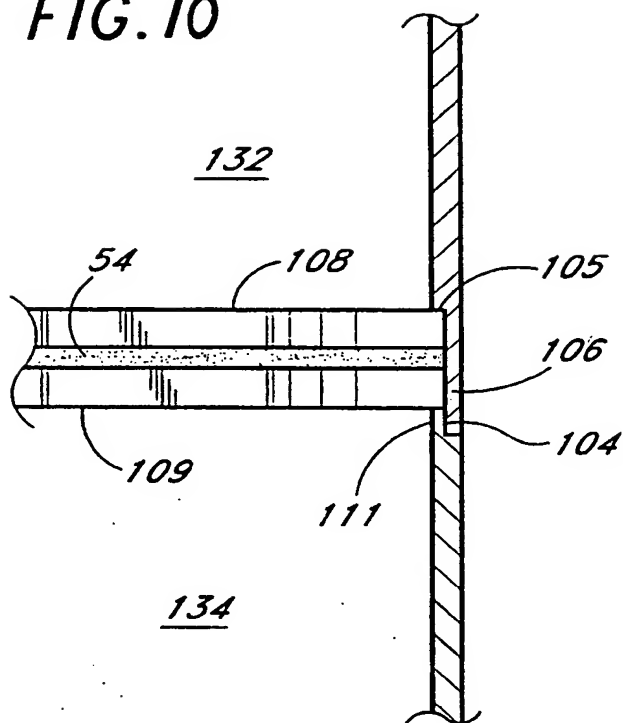


FIG. 8

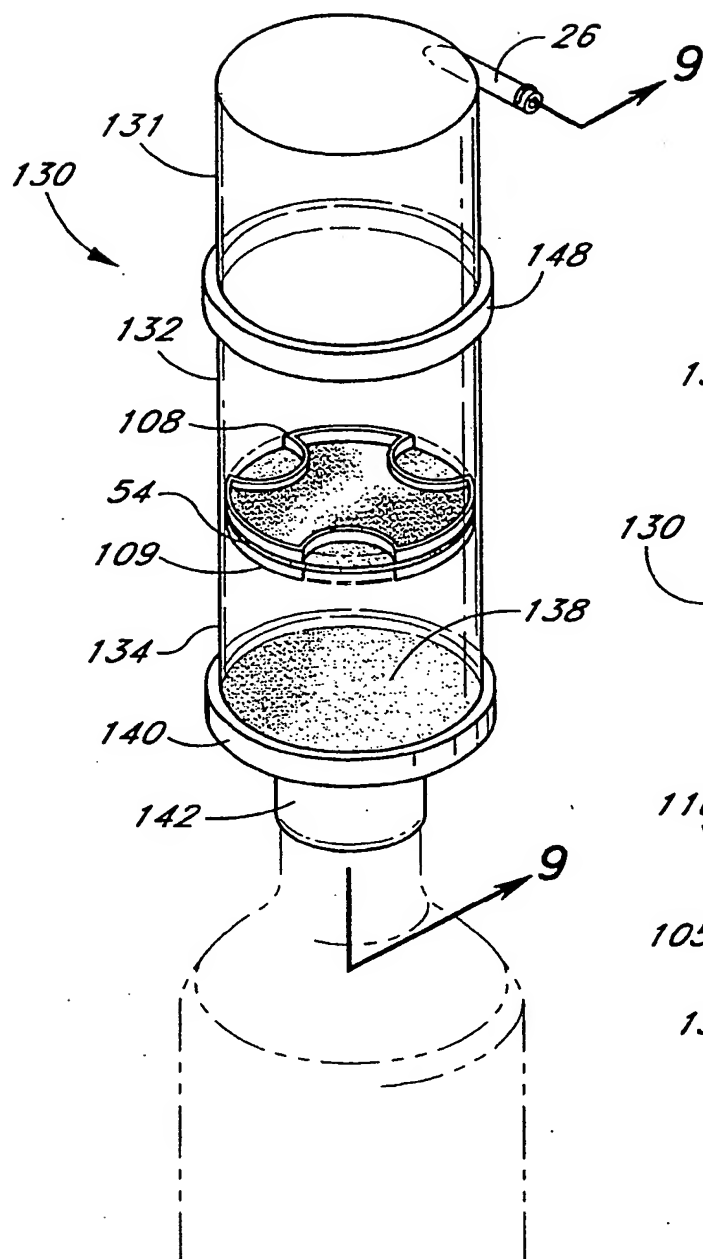


FIG. 9

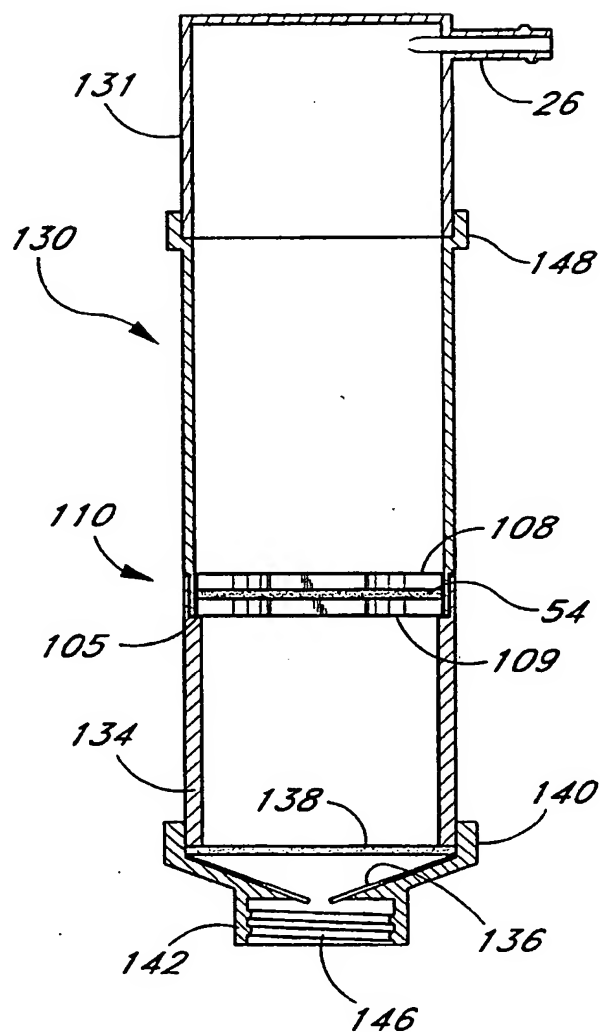
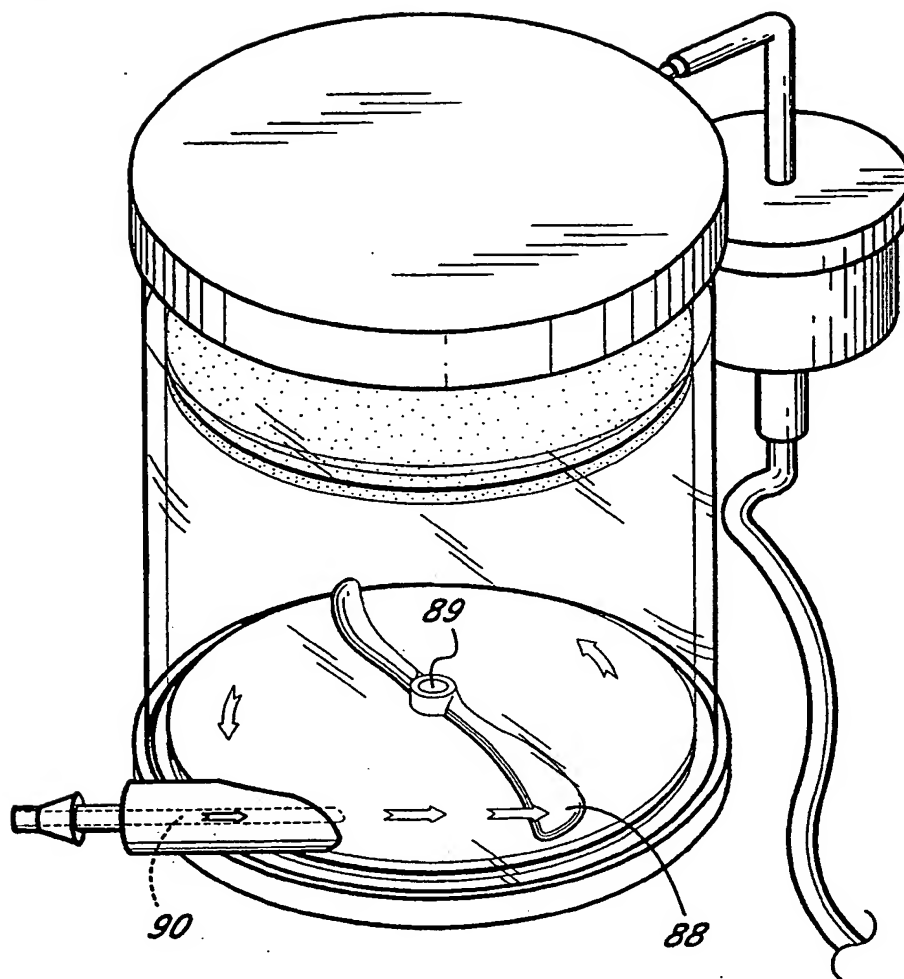
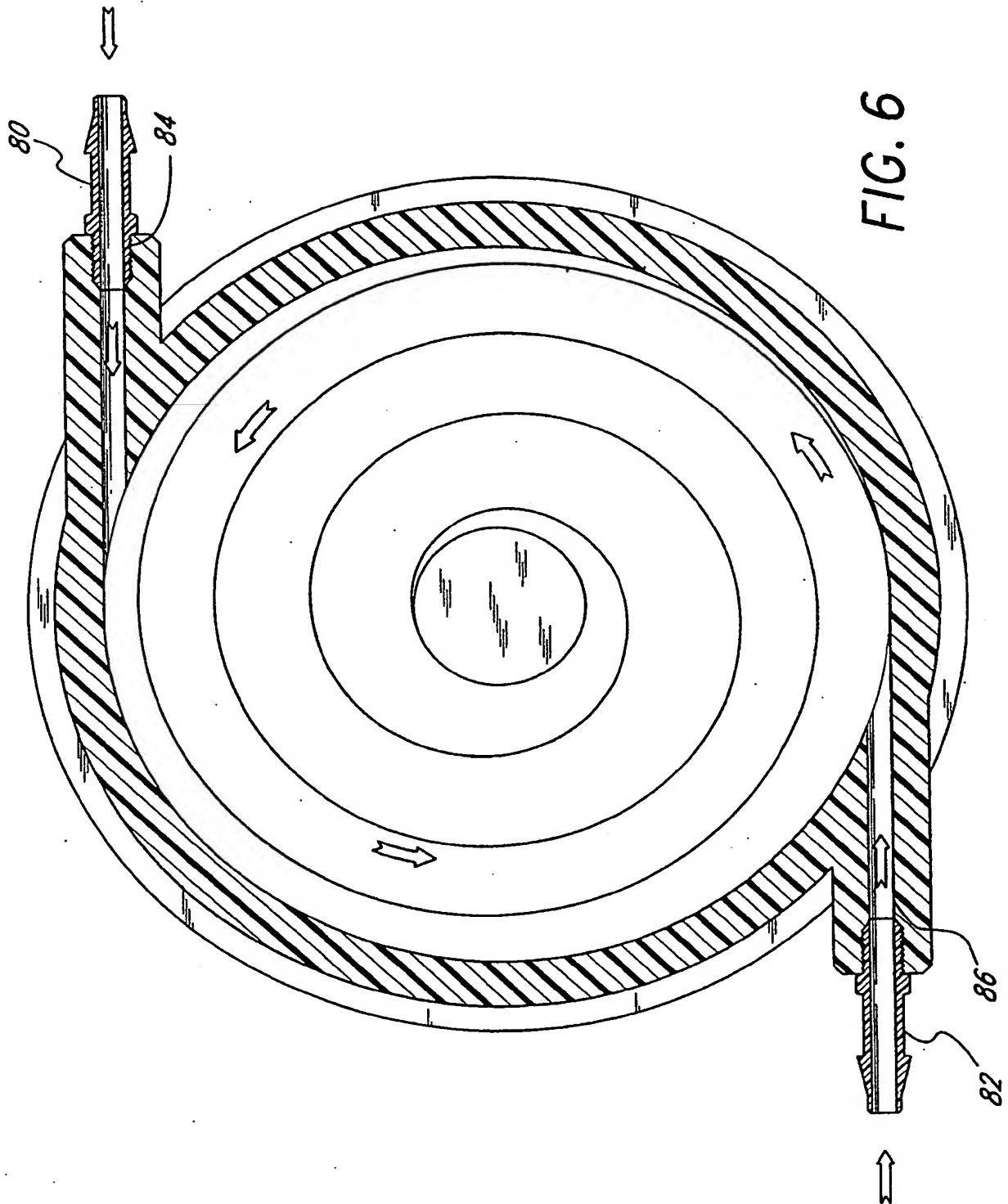


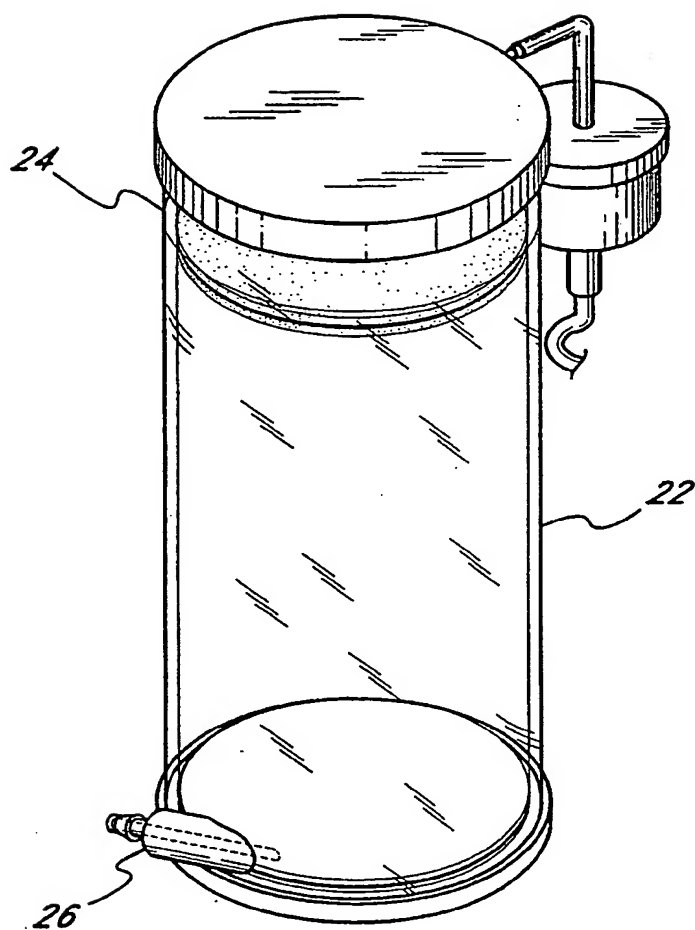
FIG. 7

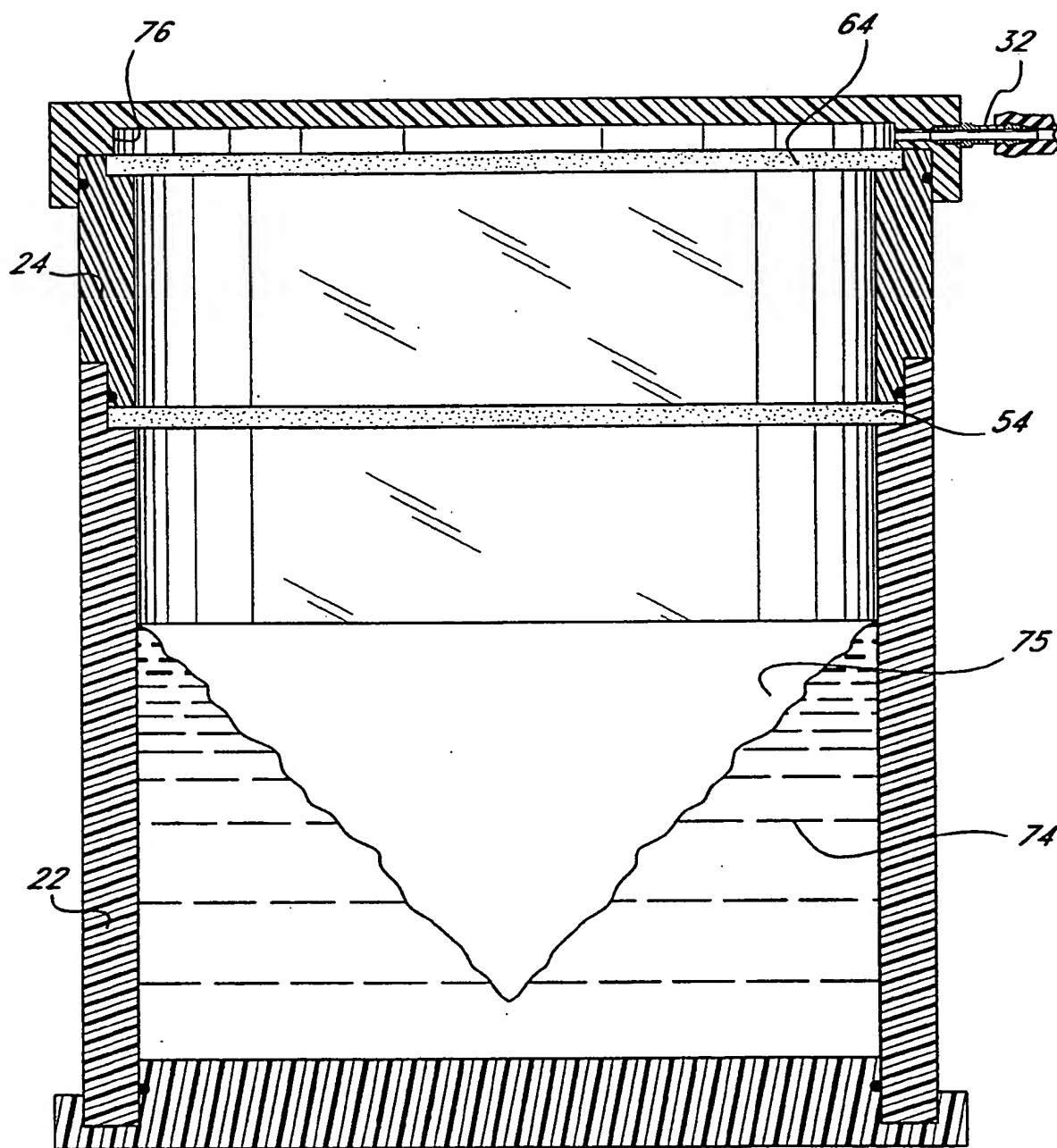




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*FIG. 5***SUBSTITUTE SHEET (RULE 26)**

*FIG. 4*

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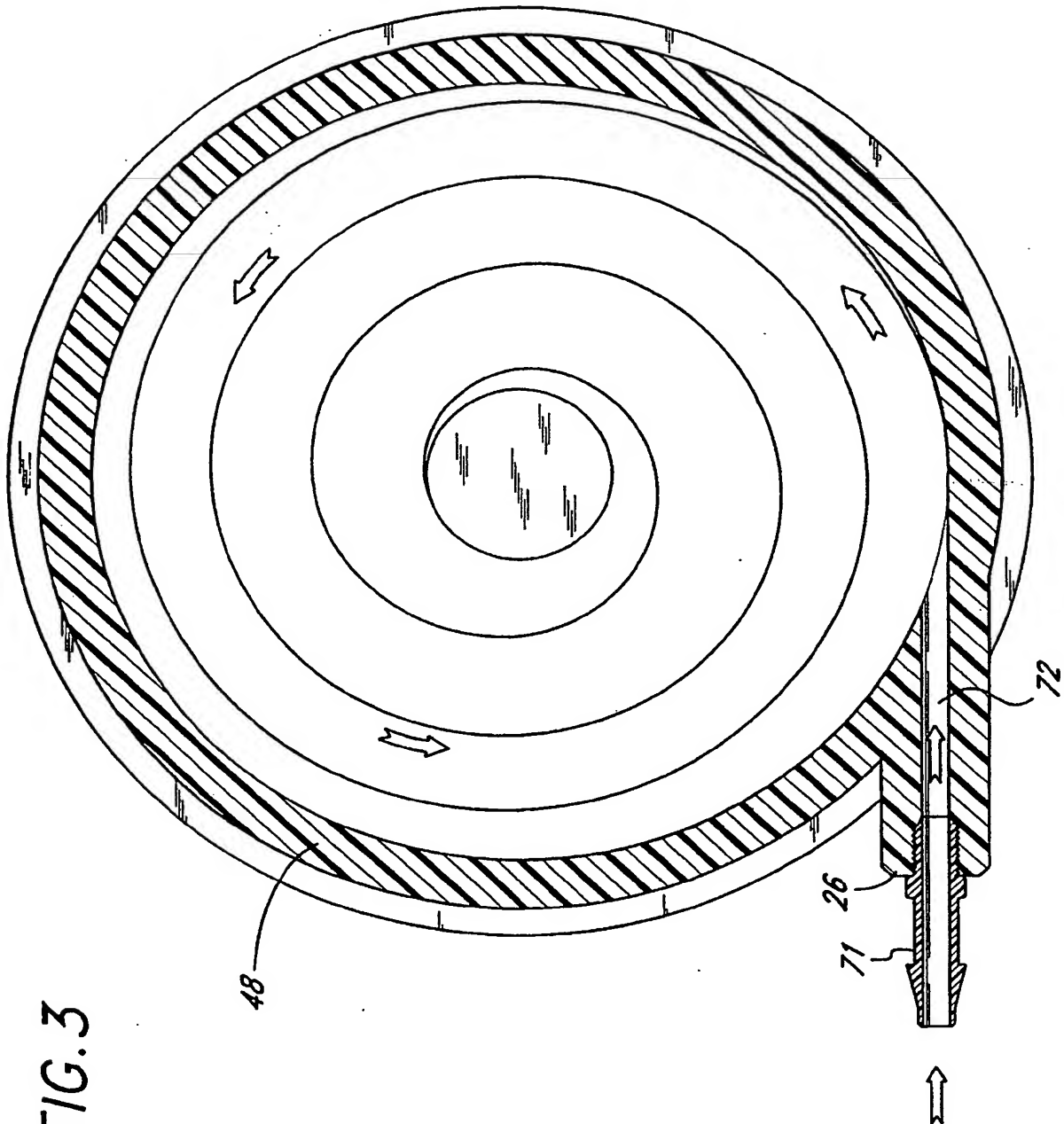


FIG. 3

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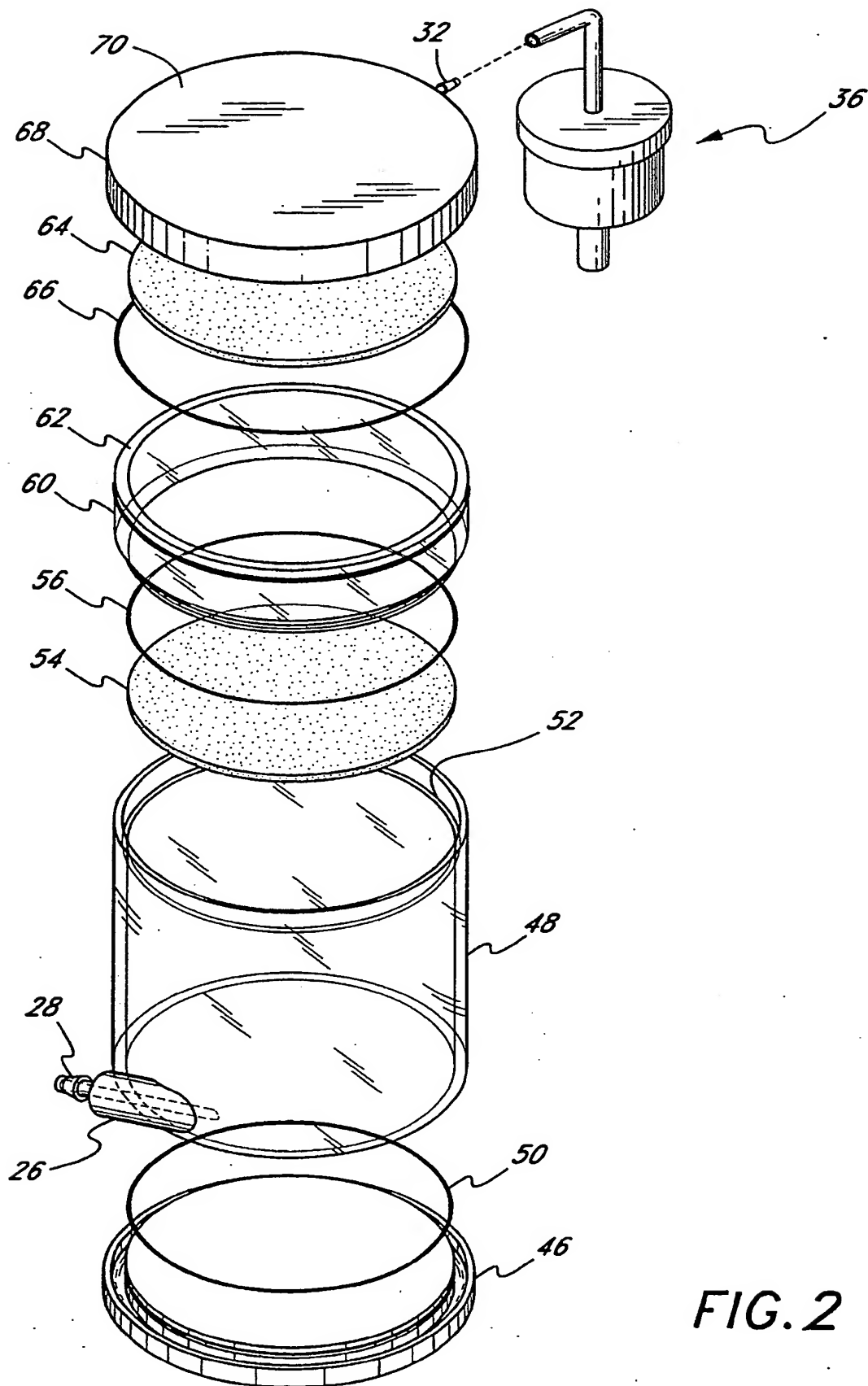
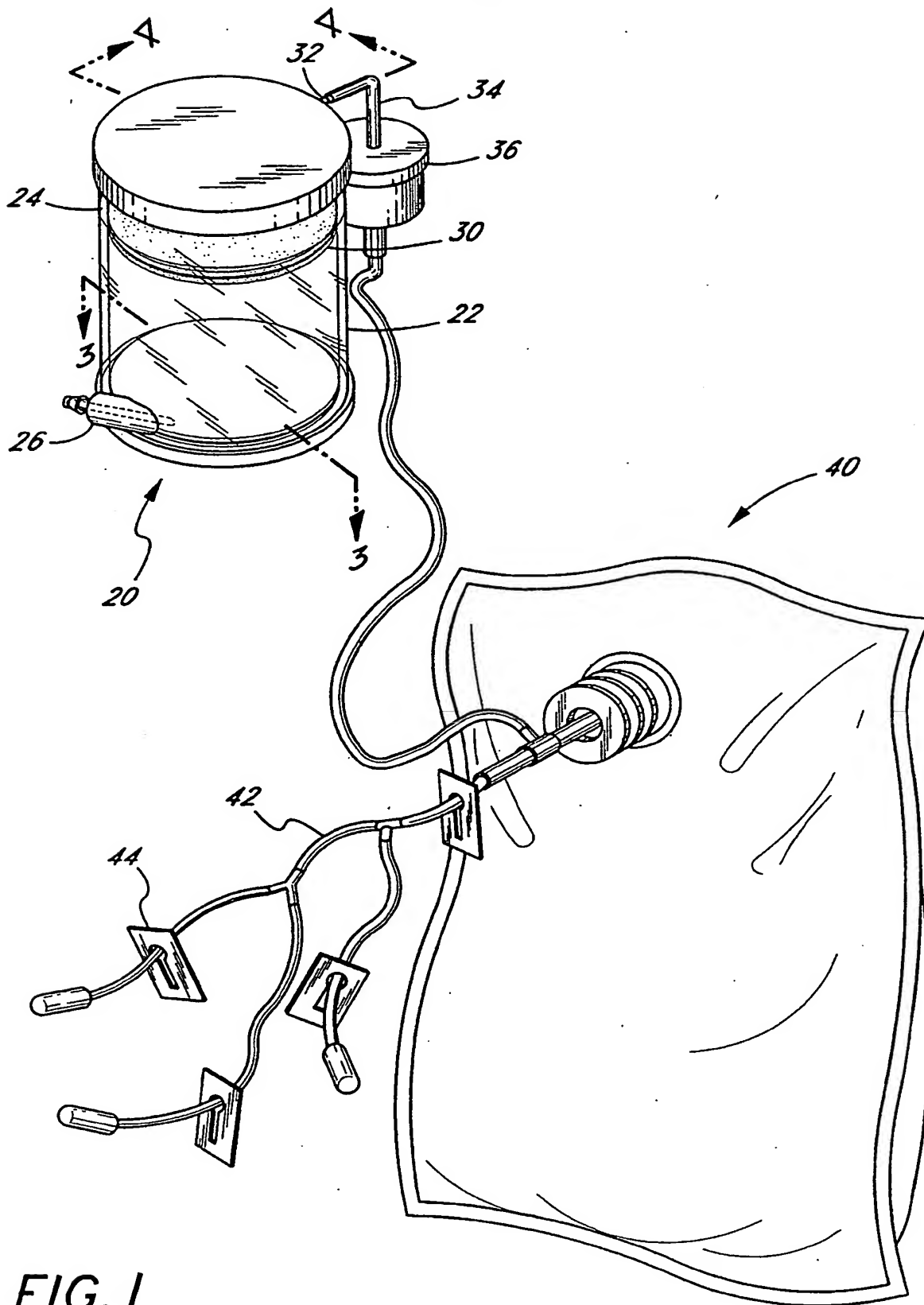


FIG. 2

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chamber, generally tangential to the interior wall of the curved base of said chamber, thereby creating a mixing vortex in the first chamber;

5 wherein the addition of a predetermined volume of the fluid under pressure produces a unit volume of fluid containing the dissolved powdered material and the additives.

20. A unit volume dissolution container as in Claim 19, wherein the additives comprise a buffer.

10 21. A unit volume dissolution container as in Claim 19, further comprising a second filter in the fluid stream, downstream of the second mixing chamber.

22. A unit volume dissolution container as in Claim 21, wherein said second filter comprises a sterilizing filter.

15 23. A unit volume dissolution container as in Claim 22, further comprising a receiving container in the fluid stream, downstream of the sterilizing filter, for receiving the unit volume of fluid containing the dissolved powdered material and the additives.

20 24. A unit volume dissolution container as in Claim 19, wherein said first filter between said dissolution chambers is held in place by at least one filter support structure.

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introducing an influent fluid stream under pressure into the first dissolution chamber for contacting the powder and creating a vortex therein; and

5 directing the fluid containing the dissolved first powder into the second dissolution chamber for contacting the second powder thereby generating an effluent fluid stream containing the first and second dissolved powder.

10 16. The method of Claim 15, further comprising the additional step of inverting the apparatus periodically during the dissolution process.

17. The method of Claim 15, further comprising the additional step of periodically increasing the back pressure in the apparatus during the dissolution process.

15 18. The method of Claim 17, wherein the increasing the back pressure step comprises obstructing the effluent stream.

19. A unit volume dissolution container for reconstituting a unit volume of powdered material in a fluid, comprising:

20 an elongate, generally cylindrical housing having first and second ends and a fluid flow path extending therethrough;

25 a first dissolution chamber in the flow path and proximate the first end of the housing, said first dissolution chamber having a curved base and containing a sufficient quantity of powdered material to produce a unit volume of reconstituted material;

30 a second dissolution chamber in the flow path and proximate the second end of the housing, said second dissolution chamber containing a sufficient quantity of additives to produce a unit volume of reconstituted material;

35 a first filter between said first and second dissolution chambers for maintaining separation of the powdered material and additives until the addition of the fluid; and

an influent port in the housing, for directing a pressurized fluid stream into the first dissolution

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a connector in fluid communication with the effluent port, said connector adapted to releasably engage a fluid receiving receptacle; and

5 a first filter located between said dissolution chamber and said effluent port.

6. The apparatus of Claim 5, wherein said filter is a sterilization filter.

7. The apparatus of Claim 5, wherein said filter is a porous filter disk.

10 8. The apparatus of Claim 5, further comprising a second dissolution chamber in the flow path, said second dissolution chamber proximate the second end of the housing and separated from said first dissolution chamber by said filter.

15 9. The apparatus of Claim 8, wherein a second filter is positioned between said second dissolution chamber and said effluent port.

10. The apparatus of Claim 8, wherein said second filter is a sterilization filter.

20 11. The apparatus of Claim 5, wherein said filter is supported by at least one filter support structure.

12. The apparatus of Claim 5, wherein said connector is adapted with threads to screw onto a fluid-receiving receptacle.

25 13. The apparatus of Claims 5, wherein said connector is adapted with a twist-lock mechanism to lock onto a fluid-receiving receptacle.

30 14. The apparatus of Claim 5, wherein said first dissolution chamber comprises a top and bottom section, wherein said top and bottom sections are releasably engaged with one another.

15. A method for dissolving at least two powders in a fluid, comprising the steps of:

35 providing a vortex dissolution apparatus having a first powder in a first dissolution chamber therein and a second powder in a second dissolution chamber therein;



WE CLAIM:

1. A method for dissolving at least one powder in a fluid, comprising the steps of:

5 providing a vortex dissolution apparatus removably secured to a fluid-receiving receptacle, said apparatus having at least one chamber containing at least one powder and at least one filter housed therein;

10 introducing an influent fluid stream under pressure into the first dissolution chamber for contacting the powder and creating a vortex therein; and

directing the fluid from the first dissolution chamber through the filter and into the fluid-receiving receptacle.

15 2. The method of Claim 1, wherein said powder is tissue culture media.

3. The method of Claim 2, wherein said apparatus has two chambers, each chamber housing powder and wherein said filter is a sterilization filter.

20 4. The method of Claim 3, wherein said receptacle is a bottle.

5. A unit volume dissolution apparatus for preparing a unit volume of powdered material in a fluid, comprising:

25 an elongate, generally cylindrical housing having first and second ends and a fluid flow path extending therethrough;

30 a first dissolution chamber in the flow path and proximate the first end of the housing, said first dissolution chamber containing a sufficient quantity of powdered material to produce a unit volume of dissolved material;

35 an influent port in the housing, for directing a pressurized fluid stream into said first dissolution chamber, generally tangential to the interior wall of said chamber, thereby creating a mixing vortex in said chamber;

an effluent port associated with the second end of the housing;

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combination of a closed system with desiccant under vacuum yields prepackaged units having a relatively long shelf life and improved tolerance to temperature change over the corresponding liquid product.

5           The invention disclosed herein has numerous applications and while particular embodiments of the invention have been described in detail, it will be apparent to those skilled in the art that the disclosed embodiments may be modified given the design considerations discussed herein. Therefore, the  
10           foregoing description is to be considered exemplary rather than limiting, and the true scope of the invention is that defined in the following claims.

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have flow paths of varied diameter to direct the majority of fluid into the first chamber and less fluid into the second. This promotes vortex formation in the first chamber during the simultaneous reconstitution of both chemicals.

5           While the preferred embodiments described herein employ powdered chemicals, it is contemplated that the mixing apparatus of the present invention will work equally well for the reconstitution of a concentrated liquid or a sequential combination of liquid and powder.

10           More viscous solutions or chemicals with reduced solubility may require some externally powered mechanized mixing. Magnetic stir bars can be provided in either the lower or upper chambers to facilitate mixing when the apparatus is placed on a magnetic stir plate. Further, a  
15           motor driven impeller can be provided for connection to a motor to create a vortex of sufficient strength to reconstitute the dry powder.

          Thus, in an additional embodiment a mechanized impeller or other internal rotation device is used to provide a  
20           rotational force to generate sufficient liquid turbulence to reconstitute the chemical contained in the self-contained unit dose reconstitution system disclosed herein. If sufficient mixing force can be generated by the motor driven impeller or other rotational device then the fluid need not enter the  
25           chamber at a tangential angle and, where more than one influent port is required, these ports need not be aligned in the same vertical or horizontal plane.

          Thus, the invention disclosed provides a method and apparatus for the single step preparation and, if required, sterilization of a given chemical. The system is closed,  
30           therefore handling is minimized. All chemicals are premeasured so employee efficiency is maximized. The closed system additionally permits a complex sequential or multicomponent reconstitution and sterilization process to be  
35           performed in a convenient location without the risk of contamination and with minimal variation in end product due to technician error or batch variation. In addition, the

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be able to be readily optimized by one of skill in the art for the reconstitution of a given formulation.

5 In accordance with a further embodiment of the present invention, a second water-driven mixing chamber is provided by directing the effluent from the first chamber through an orifice aligned along a tangent to the interior wall of a second generally cylindrical chamber. In this embodiment, the same influent stream is used to sequentially drive two successive vortex mixing chambers in series relationship where  
10 chemical B requires some agitation to dissolve.

In accordance with another embodiment of this invention there is provided a mixing apparatus wherein the influent stream is divided into two or more parallel flow paths before entering the first mixing chamber and each flow path is  
15 directed to a separate mixing chamber. In this embodiment, two or more mixing chambers are provided in parallel fluid flow relationship, each with separate chemical contents such that two or more chemicals can be individually and simultaneously reconstituted. It is further contemplated that  
20 the plurality of multiple mixing chambers could be maintained as separately reconstituted units, or the effluent streams can be recombined to produce a single volume of reconstituted product. Physically, the plurality of mixing chambers can either exist as separate structures, or combined together such  
25 that each mixing chamber comprises a separate chamber within a common housing.

For example, in a modification of the embodiment depicted in Figure 5, the influent stream is divided to provide an influent stream through influent port 26 and also through a  
30 second influent port (not illustrated) tangentially aligned to the interior wall of chamber 24.

In this embodiment, mixing of chemical A with chemical B can occur after both chemicals are reconstituted by elimination of fluid communication directly between the two  
35 chambers. It is further contemplated that the influent stream can be divided unequally between the multiple chambers. In this example, the fluid dividing fork or influent ports may

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above. For example, turbine-like stirring blades added to the lower chamber could add additional turbulence. Referring to Figure 7, stirring blades 88 are freely rotatable around a central axis 89. Fluid entering influent port 26 initiates rotational movement of blades 88 and blade rotation supports increased turbulence within the chamber and provides a fluid rotation guide for additional incoming fluid. In the illustrated embodiment, the axis of influent port 90 is aligned to direct an incoming stream directly against the blades 88. Alternatively, blades 88 can be provided in the embodiment illustrated in Figure 2 or 6 having a tangential flow alignment.

In an alternative water-driven mixing embodiment, the influent fluid stream is first directed through an external turbine located outside of the mixing chamber, preferably within a separate turbine chamber. The force of the liquid under pressure initiates the rotation of the external turbine blades and rotation is maintained by the velocity of additional liquid entering the apparatus. The liquid effluent leaving the activated turbine blades is thereafter directed through a tangential influent port or other influent port leading to the mixing chamber.

Liquid entering the mixing chamber from the turbine chamber contacts a set of mixing blades which may be similar to the blade system illustrated in Figure 7. These blades are driven by the rotational energy from the turbine chamber blades and preferably also by the tangential inflow of the influent liquid under pressure.

This invention discloses a number of embodiments that provide a closed, self-contained mixing system to reconstitute a unit dose of chemical into a known final liquid volume. The discussion provided above serves to point out those design features that can be modified to adapt the disclosed apparatus for a wide range of applications. The desirability of specific influent port angles, position, number and diameter along with chamber dimensions, fluid pressure and a need for external turbulence generators are design features which will

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influent flow pressure to create fluid turbulence then the  
influent ports 80 and 82 are preferably both aligned  
tangentially to the interior surface of the first chamber.

5 In the illustrated two-part embodiment, influent ports 80  
and 82 have equal port diameters 84 and 86. The diameters may  
be individually modified for varied influent flow velocities.  
Further, the inflow ports should be positioned so that the  
inflow from port 80 does not interfere with the inflow from  
10 port 82. The arrows illustrated in Figure 6 indicate that  
fluid tangentially entering the mixing chamber from both ports  
flows in tandem to maintain vortex activity.

The second influent port could alternatively be situated  
in the same vertical plane as the first influent port. Fluid  
entering the second port at a sufficient velocity assists the  
15 vortex created by fluid entering from the first port. For the  
reconstitution of large amounts of dry powder or viscous  
solutions, two influent ports might better facilitate complete  
mixing. Thus, water or other solvent could be added from more  
than one influent port solely to support vortex generation.  
20 Alternatively, the liquids entering the apparatus through  
multiple influent ports could be of different chemical  
composition.

Where multiple ports are used, the interior diameters of  
each of the ports and influent pressures can be varied to  
25 promote mixing of the desired reagents. A smaller diameter  
port situated above a larger diameter port would provide  
additional inflow velocity over the larger diameter port. In  
this way an efficient vortex could be maintained to maximize  
reconstitution of a given powder mixture. These design  
30 features will be added or included depending on the solubility  
of the powder in a particular application, the volume of  
powder relative to the chamber size and by the chemistry  
required to reconstitute a given liquid preparation.

If additional turbulence is required to reconstitute one  
35 or more of the chemicals, additional water-driven stirring  
means may be added to facilitate mixing either instead of or  
along with the tangential inflow vortex mixing discussed

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necessarily have to be positioned on top of one another. It is contemplated that the two chambers could be disposed side by side or remote from one another and in fluid communication by way of silicone, glass or other conventional tubing.

5            Depending upon the chemistry of a given system, a single mixing chamber may be all that is required. Alternatively, more than two chambers could additionally be linked in succession within the same tubular housing for the sequential dissolution or reconstitution of more than two chemicals.

10          Each chamber is typically defined by a chamber divider, preferably a filter, such as the microporous filtration disc located between the first and second mixing chambers of the preferred embodiment shown in Figure 2. This would prevent undissolved solids from passing between chambers. The

15          chambers may be all contained within a single housing or provided as individual remote units. These are linked in succession with tubing or other connection devices known to those in the art.

          It is also contemplated that other applications for the

20          disclosed invention may require the apparatus to have more than one influent port. There are chemical mixtures that require the simultaneous addition of two or more solvents for reconstitution of a given powder or concentrate. For example, the preparation of chemicals containing EDTA (ethylenediamine

25          tetraacetic acid) using the disclosed apparatus could require two influent ports. The disodium salt of EDTA will not go into solution until the pH of the solution is approximately 8.0. Therefore, the preparation of a buffer containing EDTA could require an influent port for water and an additional

30          port for a NaOH solution to fully dissolve the powder contained in the provided chamber.

          The influent ports can be positioned on the same horizontal plane, along the same vertical plane, or elsewhere, depending upon particular requirements of a given application.

35          Figure 6 provides a cross-sectional view of a mixing chamber embodiment having two influent ports 80 and 82 positioned along the same horizontal plane. If mixing relies solely on

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industrial applications. For example, many liquid pharmaceuticals are prepared in the hospital pharmacy with some frequency and quantity. Saline solutions, alimentary preparations, imaging reagents, dyes, sterilization solutions and anesthetics are reconstituted as liquids. Premeasured aliquots provided ready for reconstitution such as contemplated by the disclosed invention would provide an advantage over the current art.

Alternative applications include, but are not limited to, preparation of pesticides, fertilizers, any of a variety of beverages commonly prepared from powder such as milk, iced tea, etc. which could all be reconstituted using the disclosed invention. It is further contemplated that the liquid solvents employed by this invention could be water, alcohols or other organics. The solubility characteristics, the solvent to be used, the amount required and the chemical interactions between the solvent and the reconstituted chemicals will serve to provide guidelines for the size of the mixing chamber and the choice of materials for the components as described in association with Figure 2. In addition, the applications contemplated for Figure 2 can advantageously be used in the embodiment associated with Figures 8 and 9.

A variety of modified forms of the invention can be constructed for different end uses. For example, the diagrams depict a preferred embodiment wherein the first mixing chamber is coaxially aligned beneath the second chamber and separated by a microporous circular filter disc. In this embodiment the upper and lower chambers both have a cylindrical shape and the circular filter disc follows the shape of the chamber casing. As noted, the lower chamber preferably has a generally cylindrical shape in order to facilitate rotational fluid velocity of sufficient turbulence.

However, it is not necessary for the upper chamber to have a cylindrical shape. Other shapes for the second chamber as well as for the microporous filter disc are contemplated. The second chamber could be rectangular, ovoid or essentially spherical. Further, the first and second chambers do not



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is removed from the hose barb influent port and tubing is attached over the hose barb. The Media Manager receptacle may be placed on a scale and the mixing chamber device is placed upright on a solid surface.

5           Water is directed through the apparatus, through the chambers and sterilization filter, and reconstituted media flows into the Media Manager receiver. During operation, the water flow is turned off occasionally for about five seconds each time to relieve pressure in the system. When the  
10       receiver has been filled, an aliquot is tested for pH and HCl may be added through one of the multiple inlet ports to reach a desired endpoint pH of within the range of from about 6.8 to about 7.5. In addition, other amino acids, other buffers (i.e., HEPES  $C_6H_{13}N_2O_4S$ ) or supplemental glucose can be added  
15       through multiple inlet ports 42.

          The receptacle is disconnected from the sterilization filter and capped, and the receptacle is inverted briefly or agitated to mix the contents before use. The media can be used directly for large batch tissue culture or can be  
20       aliquoted into smaller volumes if desired.

          The above examples describe the use of the disclosed invention for the reconstitution of Minimum Essential Media for tissue culture. There are numerous other tissue culture medias that could be prepared using the disclosed apparatus.  
25       These include but are not limited to F-10 Nutrient Mixture (Ham), Dulbecco's Modified Eagle Media (DME), and RPMI Media 1640. It is contemplated that a custom media could additionally be supplied in the above mixing chamber or that a variety of other laboratory chemicals and buffers could be  
30       provided for commercial use. Bacterial growth media could also be provided in the disclosed apparatus.

          Certain laboratory reagents are used in large scale. Tris-acetate buffers, Tris-borate buffers, or glycine based electrophoresis buffers could be provided in the contemplated  
35       mixing chamber apparatus together with a filtration device.

          It is additionally contemplated that the apparatus disclosed herein has a number of other commercial or

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sterilization filter with a 10-liter Media Manager receiving receptacle (Irvine Scientific, Santa Ana, CA).

During assembly of the mixing chamber, MEM powder having a granulation size of about 70-120 micron is added to the lower chamber and powdered sodium bicarbonate is added to the upper chamber. MEM powder can be purchased as a prepared powder from Irvine Scientific or the individual ingredients can be purchased from chemical suppliers known to those with skill in the art. The quantity of each component to prepare 10 liters of a typical MEM formulation at a 1X concentration are provided below.

<u>Component</u>	<u>Amount (g)</u>	<u>Component</u>	<u>Amount (g)</u>
CaCl <sub>2</sub>	2.0	KCl	4.0
MgSO <sub>4</sub>	2.0	NaCl	68.0
Na <sub>2</sub> HPO <sub>4</sub>	1.4	D-Glucose	10.0
Phenol Red	0.1	L-Arginine	1.26
L-Cystine	0.24	L-Glutamine	2.92
L-Histidine	0.42	L-Isoleucine	0.52
L-Leucine	0.52	L-Lysine HCl	0.72
L-Methionine	0.15	L-Phenylalanine	0.32
L-Threonine	0.05	L-Tryptophan	0.10
L-Tyrosine	0.36	L-Valine	0.46

and 10.0 mg of each D-Ca pantothenate, Choline chloride, Folic Acid, Nicotinamide, Pyridoxal HCl, and Thiamine HCl. 20 mg I-inositol and 1.0 mg Riboflavin are additionally added.

Twenty-two grams of Sodium Bicarbonate are placed in the upper chamber.

The foregoing are all provided in a closed system comprising the mixing chamber, tubing, sterilization filter and Media Manager receiving receptacle to the user in packaged form under vacuum, with desiccant.

#### EXAMPLE 2

To use, the filled apparatus of Example 1 is removed from its packaging. Additional tubing is attached to a double deionized water source (preferably tap ddH<sub>2</sub>O, or alternately a water source associated with a pumping apparatus). No special equipment or sterile technique is required. The cap.

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flexible bag such as the Media Manager Product (Irvine Scientific, Santa Ana, California).

5 In a preferred application of the invention, the chemical A is powdered tissue culture media such as DME, available from Irvine Scientific, Santa Ana, California, and chemical B is sodium bicarbonate ( $\text{NaHCO}_3$ ) and/or other appropriate buffers or additives depending upon the media. Reconstituted, buffered tissue culture media enters receiving receptacle 40 as shown in Figure 1.

10 Multiple inlet ports 42 may also be used to supply additional additives such as HCl or NaOH to adjust the pH of the reconstituted media. Glutamine and additional buffering agents may also be added through these ports. The final product is mixed by shaking the receptacle 40 and used  
15 directly out of receptacle 40 or aliquoted into additional sterile vessels.

The following are preferred embodiments of the disclosed apparatus illustrating the use of the mixing chamber device together with a sterilization filter and holding receptacle  
20 for the reconstitution of tissue culture media.

#### EXAMPLE 1

The mixing apparatus is designed for the reconstitution of 10 liters of Eagles Minimum Essential Medium (MEM). The overall configuration of the apparatus can be observed in  
25 Figure 1. The apparatus is provided as a cylindrical dual chamber system having lower chamber dimensions of 4.5" diameter X 4" height, and upper chamber dimensions of 4.5" diameter X 1.5" height. The influent port has a cross-sectional diameter of 3/16". Upper and lower mixing chamber housings are molded from polystyrene. The mixing chamber base and mixing chamber cap are molded from polypropylene and for this particular embodiment, a 0.25-inch air space is provided between effluent filter 64 and the interior surface of the  
30 mixing chamber cap. Flexible silicone tubing connects a nylon sterilization filter obtained from Pall Corporation to  
35 effluent port 32. Sterile silicone tubing connects the

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believed to force water between the powder grains thereby displacing air. This results in greater water/powder contact yielding an increased rate of dissolution.

5        There are a number of materials that could be used for  
the manufacture of the mixing chamber apparatus of Figure 2  
and in general, of this invention. The choice of materials  
will be dictated by the choice of solvent and chemical  
destined for reconstitution. To avoid chamber and solvent  
10       reactivity, chamber materials and sealing devices should be  
relatively resistant to solvent degradation. The choice of  
chamber materials and sealing mechanisms could additionally be  
dictated by thermal considerations depending upon the  
reactivity of the solvent with chemical A or B. Thus,  
15       chemicals initiating intense exothermic reactions should  
typically not be placed in a mixing apparatus, for example,  
sealed with heat sensitive glue. The choice of materials,  
solvents, and chemicals for functional mixing chamber assembly  
will be apparent to those with skill in the art. The  
20       materials listed above are exemplary and should in no way be  
construed as limiting upon the invention disclosed herein.

      If a sterile reconstituted product is required, then a  
sterilization exit filter apparatus is preferably provided.  
The sterilization exit filter can either be internal.  
Preferably a 0.2 $\mu$  pore size filter is used. In addition, the  
25       filter should be able to accommodate the desired fluid flow  
rates, such as between about 2-5 liters/min. Preferably the  
filter housing contains a manual vent and the housing is  
translucent so that the user is able to determine if the unit  
is filling with air.

30       Flexible tubing for providing communication between  
system components may be sterilized, such as by autoclave or  
gamma irradiation, and assembled together at the point of  
manufacture. It is additionally preferred that a sterile  
receiving receptacle be supplied with the apparatus. The  
35       sterile receiving receptacle could be glass, plastic, or metal  
and could be preformed or flexible. In a preferred  
embodiment, the receiving receptacle comprises a sterile

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velocity. Further, the preferred design of Figure 11 accommodates a wide range of powder volumes. This embodiment functions efficiently even when the powder volume approaches 100% of capacity.

5        Like the embodiment of Figure 2, conventional tubing is utilized to direct water from the water source to the influent port 208 of the apparatus illustrated in Figure 11. For standard powder dissolution applications the tubing preferably has an internal diameter of about 1/4" - 1/2". Housings  
10       provided with sanitary connectors on the influent or effluent ports may require sanitary to hose barb reducing adapters. For 1-1/2" sanitary connectors employing hose barb reducing adapters, the adapters preferably have an internal diameter of  
15       at least about 3/8". The tubing connected to both the influent and effluent port is preferably secured with spring clamps, adhesives, ring clamps, ty-wrap connectors or the like.

      There are a wide range of variables that will determine how rapidly a powder will dissolve in this apparatus. For  
20       example, the granule size of the powder, the volume of powder, the chemical composition of the powder, the water temperature and the rate of flow of water into the device will all influence the time required for total powder dissolution.

      The ability of the apparatus to reconstitute powder  
25       within a given time can be improved by briefly interrupting the fluid inflow at repeated intervals to generate a pulsed flow. A preferred method for achieving interrupted flow is to simply pinch off the outlet tubing attached to the effluent nozzle between the mixing apparatus and the sterilizing  
30       filter. A periodic two to three second interruption is sufficient for improving the dissolution activity of this apparatus. The apparatus may be inverted occasionally during operation to additionally reduce the time required for total powder dissolution. Any method that transiently increases the  
35       pressure within the unit can be used to improve the mixing function of the device when needed. The transient increased pressure obtained by periodically blocking fluid flow is

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Preferably, an effluent coupling 116 is affixed to effluent port 32. While there are a variety of methods recognized in the art for affixing the effluent coupling 116 to the effluent port, in a particularly preferred embodiment, the effluent coupling 116 is ultrasonically welded onto the effluent port 32.

During assembly of the apparatus of Figure 11 for use as a tissue culture media dissolution device, the effluent filter 64 is snapped into place and the upper chamber is inverted. Sodium bicarbonate or other appropriate material is weighed into the upper chamber housing 60. A first support ring 202 is seated against annular seat 212. The filter disk 204 is positioned in place, followed by second support ring 206. The powdered media is weighed into the lower chamber housing 48, and the upper chamber housing 60 is thereafter fitted with and ultrasonically welded to the lower chamber housing 48.

In one embodiment of Figure 11, designed for use as a tissue culture media dissolution device, a preferred internal diameter is contemplated to be about 3.5". A variety of different heights may be used to accommodate different volumes of solute or powders. In two preferred embodiments of this invention, the final interior heights of the apparatus are about 4" and about 16". These different heights advantageously facilitate the use of a wide range of powder volumes. The particular diameters and heights can be varied widely depending upon the intended use, dose size and other considerations that will be apparent to one of skill in the art.

Unlike the embodiment disclosed in association with Figure 2, the curved base and modified influent port of Figure 11 permit a wider range of height to diameter ratios. Preferably the height of the apparatus is greater than the diameter. Thus, the only constraints on the height to diameter ratio is that if the apparatus is too narrow, the height required to resuspend the powdered substance becomes too great. If the apparatus is too wide, the vortex mixing is lost unless there is an equivalent increase in the inlet flow

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flange 214. An axially extending annular flange 210 is configured to fit concentrically within axially extending flange 214. The flange 210 has a sufficient axial length to cooperate with the annular seat 212 and axial flange 214 to retain support ring 202, filter 204 and support ring 206 therein with either a neutral or a slight compression fit.

Preferably, the terminal end of axially extending annular flange 214 is provided with an annular bead of housing material to facilitate ultrasonic welding of the upper chamber housing 60 and lower chamber housing 48 as will be appreciated by one of skill in the art.

In the embodiment diagramed in Figure 8, the upper chamber housing 60 contains both the filter disk 204 and the effluent filter 64. A retention structure such as a radially inwardly extending annular ring 112 is preferably provided within about the top 1/16th of the upper chamber. Ring 112 is preferably formed such that the lower edge of the ring is ramped, while the upper surface forms a narrow support shoulder. In this embodiment, the mixing chamber cap 68 is generally integral with the upper chamber wall.

The foregoing structure permit installation of the effluent filter 64 by pressing the filter in the direction of mixing chamber cap 68 so that by elastic deformation the filter 64 advances past and is entrapped behind annular ring 112.

In the embodiment diagramed in Figure 8, a fluid collection space 113 is formed between filter 64 and effluent port 32. Preferably, the chamber cap 68 is conically inclined in a downstream direction to provide a space between the filter 64 and interior wall of cap 68. To prevent excessive deformation of the filter 64 due to the pressure exerted by a fluid stream, a plurality of stops such as support ribs 114 are provided. Ribs 114 are preferably affixed to the interior surface of mixing chamber cap 68, and extend radially inwardly from the upper edge chamber housing 60 toward the effluent port 32.

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be readily provided for producing a sealed container having a filter therein, as will be understood by one of skill in the art.

5       The desirability of incorporating a first support ring 202 and/or a second support ring 206 depends on a variety of operational conditions as will be appreciated by one of skill in the art. In general, depending upon the thickness and material of the filter 204, taken in combination with the  
10       desired flow rate through the filter and viscosity of the fluid flowing therethrough, the filter 204 may tend to bow slightly in the downstream direction, causing it to dislodge from its connection to the wall of the housing. Thus, a downstream support ring 202 is preferably provided to resist downstream movement of filter 204. In addition, an upstream  
15       support ring 206 is preferably also provided to insure the integrity of the assembled filter structure both during operation and shipping as will be apparent to one of skill in the art.

20       Assembly of a filter housing incorporating one or more filter support rings 202 and 206 can be accomplished in any of a variety of ways as will be appreciated by one of skill in the art. In the particular design illustrated in Figure 12, the filter 204 and support rings 202 and 206 are conveniently  
25       positioned at the junction 216 of the upper chamber housing 60 and lower chamber housing 48. In this embodiment, the radius of the interior wall of upper chamber 60 is enlarged somewhat at the junction region 216 to provide a transversely extending annular seat 212. An annular flange 214 extends axially  
30       therefrom, to produce a recess for receiving the filter 204 and support rings. In this embodiment, the first support ring 202 is positioned adjacent the annular seat 212. The filter 204 is inserted next, and a second support ring 206, if desired, is positioned adjacent the second side of the filter 204.

35       The upper edge of lower chamber housing 48 is provided with a corresponding annular recess 104 on the radially exterior surface thereof for receiving the terminal end of



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inverted cone as will be recognized by one of skill in the art. Radii that are much larger than the recited range also begin to undesirably complicate the inlet port construction.

5 The base 122 and the sidewall 124 are preferably molded as one continuous piece. Alternatively, these components can be preformed separately and secured together in any of a variety of ways known in the art.

10 Support feet 200 are preferably affixed or molded to the base 122 of chamber housing 48 to provide stability to the apparatus. The support feet 200 may comprise any number of shapes and an exemplary foot shape is illustrated in Figure 8.

15 Influent port 208 preferably comprises a coupling 28 as has been described. The influent port 208 may be manufactured as a second, separate unit from chamber housing 48. Alternatively, influent port 208 can be molded with the base 122 and sidewall 124 of dissolution chamber 48 as a single unit. In a particularly preferred embodiment, the influent port 208 is affixed to the external surface of the lower chamber housing 48 by ultrasonic welding.

20 There are a variety of designs for the influent port that are contemplated within the scope of this invention. For example, the influent port may be provided with a hose coupling to facilitate linkage to a water source. Thus, in Figure 8, the influent port 208 is provided with a hose coupling 28. In a second preferred embodiment, the influent port 208 is fitted with a sanitary fitting 118 of the type shown in Figure 13, as is well known in the art.

25 At least one support ring 202 is preferably provided for holding the filter disk 204 in place. Preferably, at least one support ring is provided on each side of filter disk 204. It is contemplated that these rings can conform to any of a variety of shapes. In general, support rings 202, 206 are preferably configured in a manner that maximizes distribution of force yet minimized interference with fluid flow. The support ring can be prepared from a variety of materials including, but not limited to polycarbonate, polyurethane and polystyrene. Any of a variety of alternative structures can

30  
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materials listed above are exemplary and should in no way be construed as limiting upon the invention disclosed herein.

If a sterile reconstituted product is required, then a sterilization exit filter apparatus 36 is preferably provided (see Figure 1). Flexible tubing for providing communication between system components may be sterilized, such as by autoclave or gamma irradiation, and assembled together at the point of manufacture. It is additionally preferred that a sterile receiving receptacle be supplied with the apparatus. The sterile receiving receptacle could be glass, plastic, or metal and could be preformed or flexible. In a preferred embodiment, the receiving receptacle comprises a sterile flexible bag such as the Media Manager Product (Irvine Scientific, Santa Ana, California).

In another preferred embodiment of this invention, a dissolution apparatus is provided with features that advantageously improve the dissolution process. In the apparatus diagramed in Figure 11, the base 122 is preferably integrally molded with sidewall 124 to form lower chamber housing 48. In this embodiment, at least the interior surface of the lower chamber 48 is provided with rounded corner 100 at the junction between the base 122 and sidewall 124.

The rounded corner 100 advantageously improves vortex formation and helps to sustain the intensity of the vortex. Rounded corner 100 forms a smooth rounded surface that helps define a pathway for the incoming fluid. In addition, the curved surface minimizes the amount of powder that can be trapped within corners or crevices within the device.

The curvature of rounded corner 100 can be varied considerably and still improve mixing over the "square" corner designs formed by a flat bottom wall on a cylindrical side wall. In general, the radius of the rounded corner 100 in a chamber having a diameter of about 3-1/2 inches will be within the range of from about 0 to about 1-3/4 inches. Larger radii can also be used, depending upon the desired functional and design characteristics. As the radius approached infinity, the lower portion of the chamber approaches the shape of an

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aligned with a macroporous filter of the type used for filter disk 54.

5 In a particularly preferred embodiment the apparatus is designed to facilitate the dissolution of powder in sufficient fluid to produce one liter of final product. In this embodiment the full size of the apparatus is about 9 cm in height with a radius of about 3.5 cm and the multi-fitting outlet is designed to fit onto a one liter fluid-receiving receptacle. In one embodiment, the powder in the first  
10 dissolution chamber is preferably tissue culture media and the powder in the second dissolution chamber is preferably sodium bicarbonate.

It is contemplated that the height ratio of the first chamber to that of the second chamber can vary widely.  
15 Therefore, in another embodiment of this invention the height of the first chamber is less than the height of the second chamber. The difference in the heights of the chambers will be determined primarily by the amount of powder to be housed in each chamber as well as by the dissolution properties of the powders. Preferably, the volume of powder in the first  
20 chamber will be less than the volume of the bottom section 132.

There are a number of materials that could be used for the manufacture of the dissolution chamber apparatus as  
25 illustrated in the figures. The choice of materials will be dictated by the choice of solvent and chemical destined for reconstitution. To avoid chamber and solvent reactivity, chamber materials and sealing devices should be relatively resistant to solvent degradation. The choice of chamber  
30 materials and sealing mechanisms could additionally be dictated by thermal considerations depending upon the reactivity of the solvent with chemical A or B. Thus, chemicals initiating intense exothermic reactions should typically not be placed in a mixing apparatus, for example,  
35 sealed with heat sensitive glue. The choice of materials, solvents, and chemicals for functional mixing chamber assembly will be apparent to those with skill in the art. The

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fluid-receiving receptacle. The apparatus is discarded and the fluid-receiving receptacle is capped to maintain fluid sterility.

5 It is additionally contemplated within the scope of this invention that the apparatus can be customized to the particular dissolution application of the user. In this embodiment, the apparatus is again supplied to the user in sterile packaging; however, the apparatus is supplied without powder. In this embodiment, the first dissolution chamber  
10 junction 148 is equipped with helical threads that threadably engage each other. The user separates the top 131 and bottom 132 sections of the first chamber 130, introduces a premeasured amount of powder or other concentrate into the first dissolution chamber and rejoins the two sections of the  
15 first chamber. If the apparatus requires the use of a second separate dissolution step, the apparatus is additionally supplied with helical threads that engage the filter housing 140 and the second dissolution chamber. The user separates the filter housing from the second dissolution chamber and  
20 adds a premeasured quantity of the second powder. In this embodiment, the filter housing 140 houses the sterile filter 138. The apparatus is reassembled and fitted onto a fluid-receiving receptacle using the same procedure described in association with the prepackaged embodiment described above.

25 In another preferred embodiment the apparatus is reusable. Here the apparatus is prepared from an autoclave resistant material and the filter housing 140 is adapted to accommodate replacement of the sterilization filter 138. Similarly, the junction between the first and second  
30 dissolution chamber can be disassociated to permit the removal and replacement of the filter disk 54.

In another embodiment of this invention, the apparatus comprises a single dissolution chamber. This embodiment is useful where two separate dissolution steps are not required.  
35 In this embodiment the first dissolution chamber is preferably directly associated with the filter housing 140 and the second filter 144 is a sterilization filter that is additionally

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the dissolution apparatus and a fluid-receiving receptacle. There are a variety of fittings that are suitable to an apparatus of the type disclosed herein. For example, the multi-fitting outlet may be designed as a pressure fitting, as a twist-lock or as a snap-lock mechanism depending on the fluid-receiving receptacle employed with the dissolution apparatus. In one preferred embodiment the multi-fitting outlet contains helical threads 146 to threadably engage a screw top glass or plastic bottle. In another preferred embodiment, the multi-fitting outlet is adapted to fit as a pressure fitting over a fluid-receiving receptacle adapted to interlock with the multi-fitting outlet. The apparatus may also include protective cap fittings (not illustrated) to fit over both the influent port 26 and the multi-fitting outlet 142.

It is also contemplated that the dissolution apparatus of Figure 8 and 9 may be provided to the user preassembled with a unit of dose of the desired powder premeasured in the apparatus, or alternatively, the apparatus can be supplied to the user without powder for customized applications.

If the apparatus is supplied prepackaged with powder that requires sterile dissolution, the user removes the apparatus from its sterile packaging and removes the protective covering over the multi-fitting outlet. The user attaches the multi-fitting outlet to the mouth of a suitable fluid-receiving receptacle. Preferably, this assembly step is completed using sterile technique. The influent port 26 is then affixed to a fluid source. Fluid entering the first dissolution chamber 130 forms a vortex to dissolve the powder located therein. The fluid passes through the filter disk 54 and into the second dissolution chamber 134 where the second powder is dissolved. Fluid continues through the second dissolution chamber 134, out the effluent port 136 and into the fluid-receiving receptacle. Once the powder is dissolved and the fluid-receiving receptacle contains the desired amount of fluid containing dissolved powder, the fluid source is disconnected and the apparatus is disassociated from the

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disk is optionally included on the influent port side of the sterilization filter.

5 The second filter 138 is positioned between the second dissolution chamber 134 and the filter housing 140. In the embodiment illustrated in Figure 9, the internal surface of the filter housing 140 is ramped to direct the dissolved fluid toward the effluent port 136. The ramped surface may additionally be modified with support ribs, or the like, to maintain and support the second filter during use. The filter  
10 housing 140 is attached to the second dissolution chamber housing 134 using any number of methods known to those with skill in the art. In one preferred embodiment the filter housing is ultrasonically welded onto the second chamber housing and in another preferred embodiment the filter housing and the second chamber housing threadably engage one another  
15 such that the second filter can be replaced as necessary.

Preferably the filter housing is equipped with air outlet ports to facilitate the venting of air out of the fluid-receiving receptacle during use. In a preferred embodiment,  
20 the filter housing is equipped with air vents in the form of a plurality of small holes backed with a hydrophobic membrane to permit air to vent from the apparatus during use. In another preferred embodiment the filter housing contains a single vent port to permit gas egress during use. This vent  
25 port may take any number of forms as will be appreciated by one with skill in the art. Examples contemplated for use in this invention include, but are not limited to the air vents disclosed and described in association with U.S. Design Patent No. 270,947 to Mehra, et al. and No. 297,860 to Leoncavallo,  
30 et al. together with the air vents disclose and described in association with U.S. Patent No. 4,357,240 to Mehra, et al., No. 4,614,585 to Mehra, et al., No. 4,678,576 to Leoncavallo, No. 4,689,147 to Leoncavallo, et al. and No. 4,702,834 to Relyea.

35 The filter housing 140 is additionally equipped with a connector such as a multi-fitting housing to accommodate a variety of types of removable engagement mechanisms between

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receiving the filter 54 and support rings. The top edge of the lower dissolution chamber 134 is provided with a corresponding annular recess 104 on the radially exterior surface thereof for receiving the terminal end of flange 106.

5 An axially extending annular flange 111 extends upwardly from the wall of the lower dissolution chamber 134, and is configured to fit concentrically within at least a portion of axially extending flange 106. The flange 111 has a sufficient axial length to cooperate with the annular seat 105 and axial

10 flange 106 to retain the support rings and filter 54 therein with either a neutral or a slight compression fit. Preferably, the terminal end of axially extending annular flange 106 is provided with an annular bead of housing material to facilitate ultrasonic welding of the first chamber housing 130

15 and second chamber housing 134 as will be appreciated by one of skill in the art.

The housing of the second chamber 134 preferably terminates at its downstream end at effluent port 136 with second filter 138. For powder dissolution processes that

20 additionally require sterilization, this filter 138 is preferably a 0.2  $\mu\text{m}$  filter of nitrocellulose, or the like such as those available from Schleicher and Schuell (Keene, NH), Millipore (Millford, MA) or Nalge (Rochester NY). Other filter types and sizes are contemplated for use in this

25 apparatus and filter selection will depend on the type of fluid used for dissolution, the viscosity of the final product and the degree of sterility required.

Depending on the strength and durability of the filter material that is selected, a filter support structure may be

30 desirably included adjacent filter 138. This filter support may take any number of forms. In one embodiment the support is a support ring such as support rings 108 and 109, in another the support is a multi-ribbed plastic disk and in another embodiment, the support is a macroporous filter disk

35 such as that used for filter 54. In addition to the filter support, it is also contemplated that a macroporous filter

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140 and this filter housing is additionally adapted to include a connector such as multi-fitting outlet 142.

5 As noted, a filter disk 54 separates the first and second dissolution chambers 130, 134. Preferably filter disk 54 is a Porex filter of the type mentioned in associated with Figure 2; however, there are a wide range of other macroporous filters that are commercially available. This filter is preferably supported by at least one support ring 109. In the embodiment illustrated in Figure 8, two support rings 108 and 109 are used to support the filter disk from both sides of the filter. It is contemplated that these rings can conform to any of a variety of shapes. In general, support rings 108 and 109 are preferably configured in a manner that maximizes distribution of force yet minimizes interference with fluid flow. The support rings can be prepared from a variety of materials including, but not limited to polycarbonate, polyurethane and polystyrene.

20 Assembly of the filter with one or more filter support rings 108 and 109 can be accomplished in any of a variety of ways as will be appreciated by one of skill in the art. In the particular design illustrated in Figure 9, the filter 54 and support rings 108 and 109 are conveniently positioned at the junction of the first chamber 130 and the second chamber 134.

25 Referring to Figures 9 and 10, there is disclosed one embodiment of adjunction between the lower portion 132 of upper chamber 130 and lower chamber 134. The relative positions of the upper and lower corresponding surface structures can be readily reversed. Alternatively, any of a variety of connection configurations can be used to assemble the mixing chamber, as will be apparent to one of skill in the art.

35 In the illustrated embodiment, the radius of the interior wall of the lower section 132 of dissolution chamber 130 is enlarged somewhat at the junction region to provide a transversely extending annular seat 105. An annular flange 106 extends axially therefrom, to produce a recess for



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top chamber section 131. These sections are joined at the first chamber junction 148.

From the first dissolution chamber, the fluid passes into a second dissolution chamber 134. The first and second  
5 dissolution chambers are separated by a filter disk 54. From the second dissolution chamber, fluid passes through a second filter 138 and into the filter housing 140. The filter housing 140 focuses the fluid through the effluent port 136 and out of the apparatus through the multi-fitting outlet 142.  
10 It is contemplated within the scope of this invention that the multi-fitting outlet is adapted to fit directly onto any of a variety of fluid-receiving receptacles such as a bottle, bell jar, erlenmeyer flask and the like.

The influent port 26 may be manufactured as a second,  
15 separate unit from the first chamber housing 130. In a particularly preferred embodiment, the influent port 26 is affixed to the external surface of the first chamber housing 130 by ultrasonic welding. In this embodiment, the first dissolution chamber housing is preferably prepared as two  
20 separate sections with the top section of the chamber 131 containing the influent port 26 and the bottom section 132 of the first chamber 130 adapted to receive the second chamber 134. The top and bottom sections 131, 132 of the first chamber 130 are joined during assembly at junction 148 such as  
25 by a weld joint, or alternatively, the top and bottom sections 131, 132 of the first chamber 130 are releasably threadably engaged with one another. The use of helical threads or other resealable connection structures instead of a weld joint permits the first chamber to be opened by the user to  
30 introduce a media to be dissolved.

The bottom section 132 of the first chamber 130 communicates with the second dissolution chamber 134 at a second junction region 110. In the embodiment of Figure 8 and Figure 9, the junction region 110 additionally contains a  
35 filter disk 54. The second dissolution chamber 134 is separated from the effluent port 136 by a second filter 138. This second filter is preferably housed in the filter housing

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as an elongate cylindrical body. However, it is additionally contemplated that the two chambers could be manufactured from different materials. Chemical B is added to the upper chamber and the upper chamber housing is similarly affixed to the mixing chamber cap having effluent port 32. The mixing chamber cap is affixed to upper chamber casing preferably in association with a rubber O-ring or other conventional sealing means.

In another preferred embodiment of the disclosed apparatus, the mixing chamber disclosed in association with Figures 1 and 2 is adapted to fit directly onto a fluid-receiving receptacle. This embodiment optionally includes a sterilization filter to sterilize the fluid effluent as it passes from the dissolution apparatus.

Figure 8 and Figure 9 illustrate a preferred embodiment of the fluid-receiving receptacle-adapted dissolution apparatus. In one embodiment, this apparatus contains two dissolution chambers and in another preferred embodiment the apparatus contains a single dissolution chamber. Referring to Figure 8, influent port 26 is adapted to accommodate incoming fluid flow at one end of the apparatus. The effluent port 136 is positioned at the other end of the apparatus.

Like the other embodiments of this invention, the influent port may contain a hose-barb connector or other means to attach to tubing, or the like, to facilitate fluid inflow. Fluid passes from the influent port 26 into the first dissolution chamber 130. While Figure 8 and Figure 9 illustrate the position of the influent port 26 at the top of the apparatus relative to the effluent port, it is contemplated that the influent port can be positioned anywhere along the first dissolution chamber 130 surface. In another preferred embodiment of this invention, the influent port is positioned near the bottom of the first dissolution chamber 130, slightly above filter disk 54.

In one embodiment the first dissolution chamber 130 is divided into two sections; a bottom chamber section 132 and a

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The manufacture of the mixing apparatus in accordance with the present invention can be accomplished using materials and techniques which will be well known to those of skill in the art. In a preferred embodiment, the mixing chamber base and cap are made of a nonreactive plastic polymer such as polycarbonate. Alternatively, the cap and base could be molded from other plastics including polysulphone. Other materials include metal alloys, plexiglass or glass.

Returning to Figure 2, the base 46 may be conveniently integrally molded with chamber housing 48. Alternatively, base 46 is assembled together with the lower chamber housing 48 to form a liquid tight seal. The lower chamber housing is preferably molded from any of a variety of materials which will remain generally non-reactive in the intended use environment, such as polystyrene, polyethylene, polycarbonate, plexiglass, lucite, polypropylene or a metal alloy. Preferably, the chamber housing 48 will be transparent to enable visual observation of its contents or the progress of the mixing cycle.

The chamber housing and the mixing chamber base are conveniently provided with a liquid tight seal through the use of an elastomeric O-ring. The first chamber can either slip fit into an annular recess on the base or threadably engage the base. The housing can additionally be sealed to the base using adhesives, a heat seal or other means known in the art.

A protective cap is provided to cover the inlet port thus preventing powder from spilling out prior to use.

During assembly of a preferred embodiment, the lower chamber is supplied with powdered media and a Porex-type microporous circular filter disc (Porex Technologies, Fairburn, Georgia) or other filter, preferably having a 90-130 micron pore size, is placed on the filter support structure. Upper chamber housing 60 is sealed to lower chamber housing 48, preferably in association with O-ring 56 or any other method for creating water tight seals. Upper chamber housing 60 is preferably made from the same material as the lower housing, and the two chamber housings may be integrally formed

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preparation of 1 liter (1), 10 l, 20 l, 50 l, and as large as 100 l or larger final tissue culture media volume are contemplated. Increasing amounts of powder in the lower chamber will require increased cylinder height and/or diameter to generate a vortex of sufficient size so as to maintain the powder in motion within the vortex until it dissolves. In addition, larger sizes may require a pump on the influent line to generate sufficient influent flow to sustain a vortex. Therefore it is contemplated that each apparatus be specifically designed to complement the final volume of product to be prepared.

Testing has determined that a powder volume greater than about 50% of the chamber volume for the powdered culture media application results in poor vortex mixing and inefficient liquid reconstitution. Testing has additionally determined that during operation of the mixing apparatus herein disclosed, improved reconstitution of the powder in the liquid is achieved by interrupting the inflow occasionally for approximately five seconds. Interrupting the flow temporarily releases pressure within the chamber thus allowing clumps of powder to draw fluid to their interior.

A precalibrated receptacle 40 can be used to determine the end point of media preparation. Alternatively, a predetermined volume of liquid can be pumped through the system or a flow meter/accumulator can be used to monitor the volume of the finished product. It is additionally contemplated that the final volume of the liquid product can be determined by weight. The receiving receptacle is placed on a scale and the receptacle is filled until the final weight of the end product is achieved.

It is important for the effective operation of the apparatus that the culture media powder remain relatively dry prior to use. Hygroscopic powders tend to clump under humid conditions and reconstitution becomes difficult. It is therefore contemplated that the commercial product comprising a mixing apparatus system with powder be packaged under vacuum and/or preferably be provided with a desiccant.

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or decreased to adjust the velocity in order to provide an adequate vortex.

5       The interior of the first chamber preferably has a substantially cylindrical configuration. This establishes a vortex guide for the liquid flow. Moreover, the cylinder diameter should complement the incoming fluid velocity. A first chamber diameter that is too large for a given influent flow will not support sufficient centrifugal force along its sides to maintain a vortex. Interior diameters that are too  
10       small could create excessive turbulence initially, but not form a vortex, thereby potentially resulting in inadequate mixing. The substantially cylindrical shape in combination with the inlet velocity and the inlet angle thus combine to set up the desired vortex.

15       Alternatively, other chamber configurations which exhibit radial symmetry may also be used for the first chamber 22. For example, spherical, hemispherical, toroidal or the like may be selected. In addition, linear-walled non-cylindrical shapes such as a frusto-conical chamber may also be used.

20       In the preferred embodiment detailed in Figure 2, the diameter of the first chamber has been found to optimally be proportional to its height. A height to diameter ratio greater than about 2.5:1 will typically not support the generation of a sufficiently strong vortex at influent flow  
25       rates of about 1-3 liters per minute.

      Figure 5 is an elevational perspective of a second embodiment of the apparatus of the present invention. Here first chamber 22 has a height significantly greater than the height of the second chamber. Under proper incoming fluid  
30       stream velocities, this apparatus could house a larger quantity of chemical A, than the embodiment disclosed with regard to Figure 2.

      In a preferred application of the invention, the mixing apparatus is used to prepare tissue culture media. It is  
35       contemplated that the mixing chamber will be provided prefilled with powdered media in a variety of unit volume sizes. For example, mixing chamber sizes to accommodate the

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Figure 3. If the rotational fluid velocity of the liquid is sufficient, the motion subsequently establishes a turbulent vortex that serves to mix the influent liquid with the contents of the first chamber.

5           Figure 4 depicts an elevational cross-sectional view of the mixing apparatus of Figure 1. The dashed horizontal lines 74 represent the swirling fluid that creates a roughly conical region of air 75 at its center. The swirling vortex mixes the contents of the first chamber 22. Additional fluid entering  
10 the chamber pushes the vortex up the sides of the first chamber and through the microporous filter disc 54 into the second chamber 24.

          Once the fluid has reached second chamber 24, the flow becomes laminar. Chemical B, located within the upper  
15 chamber, preferably has increased solubility characteristics over chemical A and therefore readily dissolves in the liquid containing chemical A. The upper chamber fills and fluid containing chemical A and B passes from the upper chamber through the effluent filter and into the cap reservoir space  
20 76. In this embodiment the effluent filter is made from the same material as circular filter disk 54. Effluent port 32 provides an outlet for the mixed product. It is alternatively contemplated that an effluent filter 64 may be deleted in which case the sterilization filter 36 could also function to  
25 trap undissolved solids.

          To create sufficient influent velocity, the liquid should enter the mixing chamber under adequate pressure to mix or dissolve chemical A. It is contemplated that slight  
30 modifications of the apparatus described in the examples provided below will be required for the proper functioning of the mixing chamber for other applications. For example, if the liquid is water and the product is tissue culture media, then normal faucet pressure, in concert with an appropriate influent port dimension will create sufficient liquid pressure  
35 to generate the desired rotational fluid velocity. The mixing chamber influent port diameter has a direct effect on inlet velocity. As noted above, the inlet diameter can be increased

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a source of fluid. In the preferred culture media application of the present invention, the influent port 26 is placed in fluid communication with a distilled deionized water (ddH<sub>2</sub>O) source having an adapted nozzle such as is found in most scientific laboratory ddH<sub>2</sub>O faucets. Other tubing materials, nozzle adapters, and pumps may be required for use with other water sources or liquid solvents.

Faucet pressure or other inflow pressures in excess of about 1 psi are generally sufficiently strong to permit proper apparatus function. Typical tap pressure, in the area of about 25 psi is sufficient for many embodiments of the invention. The minimum effective pressure is a function of the scale of the first mixing chamber, the volume of chemical A contained therein and the diameter of the influent lumen, as will be understood by one of skill in the art. Some routine experimentation may be required to optimize these parameters for specific applications. In one exemplary embodiment, utilized with an influent line pressure of about 1 to 10 psi, the first chamber is a cylindrical chamber having an interior diameter of about 4.5", an internal height of about 4", and an influent port diameter of about 3/16".

Figure 3 is a horizontal cross sectional view across plane 3-3 of Figure 1 showing a hose barb 71 connected to influent port 26. As previously described, liquid enters the lower chamber under pressure at substantially a tangent to the interior wall of the chamber. The velocity of the liquid entering the apparatus is determined by the incoming fluid stream pressure and can be additionally manipulated by altering either the diameter of the influent port or the dimensions of the first chamber. Decreased influent port diameters will increase the velocity of liquid entering the chamber, while increased influent port diameters will decrease liquid velocity. Preferably the pressure of the liquid stream in combination with a compatible influent port diameter will provide sufficient liquid velocity such that liquid entering the apparatus follows the surface of the inner chamber casing and continues along the pathway designated by the arrows of

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effluent filter 64, is placed on top of the filter support 62 following addition of chemical B. A third seal 66 is preferably used to provide a water tight seal between the mixing chamber cap 68 and the upper chamber housing. Effluent filter 64 preferably sits at least about one-eighth of an inch from the interior surface of cap 70. This provides space for liquid containing chemicals A and B to pass through the effluent filter and leave via effluent port 32.

When a sterile product is required, the fluid preferably passes through the effluent port 32 and into a sterilization unit 36. Sterilization units of the type contemplated by this invention can be purchased from a number of suppliers. One commercial supplier is Pall Corporation, Courtland, Maine. For a sterile media product, the sterilization filter apparatus will typically contain a  $0.2\mu$  filter. The filter may comprise nylon or cellulose acetate.

It is additionally contemplated that other types of filter sizes could be chosen for other functions. For example, the preparation of electrophoretic buffers requires clean, but not necessarily sterile solutions and a  $0.45\mu$  filter would be adequate. Similarly, the preparation of more viscous solutions may necessitate a wider pore size. For other applications of the invention disclosed herein, no filtration apparatus need be added. Liquid then passes directly to a receiving receptacle through flexible tubing. If a sterile filter is used, then tubing and all additional chemicals entering multiple inlet ports 42 as well receiving receptacle 40 should be sterile (see Figure 1).

In use, liquid enters the mixing chamber through influent port 26. A hose is preferably affixed to the influent port and locks in place via the hose barb connector. In a preferred embodiment, standard flexible laboratory tubing of diameter sufficiently large such that the tubing will pass over the neck of the hose barb and sufficiently small that the tubing seals over the hose barb nozzle is employed to direct the incoming fluid stream to the mixing chamber. The other end of the flexible tubing is preferably applied directly to



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a plurality of support pegs or grooves made of the same material as the cylinder casing.

5 The filter disc 54, while preferably made of Porex™ plastic (Porex Technologies, Fairburn, Georgia), could additionally be made of glass, wool, micron meshing, or any of a variety of other inert substances having suitable compatibility with the solvents and powders to be used in the apparatus. Preferably, the filter material will have a sufficiently small pore size to prevent escape of the powdered media. For the preferred application described herein, the filter preferably has a pore width of approximately 90-130 microns. The filter disk permits liquid passage into the second chamber but generally prevents the movement of undissolved solids from the first chamber 22 to the second chamber 24. Further undissolved solids trapped in the microporous filter are subsequently dissolved by the continued flow of fluid passing through the filter.

20 The two chambers are preferably adjacent one another and separated from one another by a microporous plastic filter disc 54. However, it is also contemplated that the first chamber 22 and second chamber 24 be remote from one another, so long as they can be placed in fluid communication with each other during the service cycle. Figure 2 illustrates a preferred embodiment where first and second chambers 22, 24 are axially aligned in a water tight seal such that liquid enters the first, or lower chamber, and moves to the second or upper chamber passing through circular filter disc 54. In this construction, a second seal 56 such as an elastomeric O-ring is used to provide a tight seal between the upper and lower chambers. During manufacture, chemical A is preferably placed into first chamber 22 before the circular microporous filter disc 54 has been put into place. Construction materials are discussed infra. In a preferred embodiment, lower chamber 22 is made of the same material as upper chamber 24.

35 The upper chamber housing 60 is also preferably provided with a filter support 62. A second circular filter disc, the

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a preferred embodiment enters into sterilization filter 36. Sterile liquid containing chemical A and chemical B thereafter exits filter 36 and passes into a receiving receptacle 40.

5 It is further contemplated that the final product may require the addition of one or more other liquid additives, or the receptacle 40 may be drained into a series of different containers. Therefore, multiple inlet ports generally designated as multiple inlet ports 42 are typically provided. Flow stop regulators 44 are preferably associated with each of  
10 the inlet ports to provide control for the sequential draining or influx of the desired additive solutions.

Figure 2 depicts in detail an exploded view of a preferred mixing apparatus embodiment. Mixing apparatus base 46 is combined with lower chamber housing 48 in association  
15 with a seal 50. Lower chamber housing 48 and base 46 are preferably substantially cylindrical in shape to optimize the rotational velocity of the fluid which has been driven through influent port 26 under pressure. The seal 50 is preferably an elastomeric O-ring but could be a gasket or other sealing  
20 device known to those with skill in the art.

Lower chamber housing 48 is provided with an influent port 26, generally tangentially oriented to the interior wall of the housing. Influent port 26 may be integrally molded with the housing 48, or can be affixed thereto in any of a  
25 variety of ways known in the art such as by adhesive, solvent or heat bonding techniques. Preferably, influent port 26 is located in the lower half of the housing 48, and more preferably along the lower one-fourth of the housing 48. A hose barb or other conventional connector is preferably  
30 affixed to influent port 26.

The upper inner surface of the housing 48 preferably contains an annular shoulder or support structure 52. The support structure 52 is preferably integrally molded together with or milled into the chamber housing 48 to form a ledge or  
35 lip to support a chamber divider which in this preferred embodiment is a microporous or macroporous circular filter disc 54. The support device 52 could alternatively comprise

Figure 12 is an elevational cross sectional view along the lines 12-12 in Figure 11.

Figure 13 is a cross sectional view of a sanitary connector for use on the influent and/or effluent flow ports.

5

Detailed Description of the Invention

Figure 1 is an overall system view of one embodiment of the mixing apparatus 20, filter 36 and receiving bag 40 in accordance with the present invention. The mixing apparatus 20 comprises at least one, and preferably two chambers. The generally cylindrical first chamber 22 constitutes the lower chamber in the preferred embodiment depicted herein and a second chamber 24 constitutes the upper chamber of this preferred embodiment. For descriptive purposes "chemical A" will refer herein to the material contained in first chamber 22 and "chemical B" will refer to the material contained in the second chamber 24 in a two chamber embodiment.

An incoming fluid stream enters the mixing chamber 20 through an influent port 26. The axis of the influent port enters first chamber 22 at substantially a tangential angle to the interior wall thereof such that liquid entering the first chamber through influent port 26 follows the sides of the chamber to create a circular mixing motion that facilitates mixing of chemical A with the fluid stream within the first chamber. As chemical A dissolves in the liquid and additional liquid enters into first chamber 22, the liquid level advances upward through divider 30 and enters the second chamber 24. Fluid containing chemical A passing through chamber divider 30 (Figure 1) and entering into the upper chamber now comes in contact with chemical B.

In a preferred embodiment, chemical B has increased solubility characteristics over chemical A such that agitation is not necessary to facilitate the dissolution of chemical B in liquid which already contains chemical A. Liquid containing dissolved chemicals A and B thereafter exits second chamber 24 through an effluent port 32 preferably after passing through a filter 64 (Figure 2). Liquid passing through effluent port 32 then enters outlet tubing 34 and in

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These and additional features and variations on the invention will become apparent to one of ordinary skill in the art from the detailed description of preferred embodiments which follows, when considered together with the attached drawings and claims.

#### Brief Description of the Drawings

Figure 1 is a schematic representation of the overall mixing chamber, sterilization filter, and receiving receptacle system in accordance with one embodiment of the present invention.

Figure 2 is an exploded elevational view of the embodiment of the mixing chamber and external sterilization filter illustrated in Figure 1.

Figure 3 is a top cross-sectional view along the lines 3-3 in Figure 1, showing the tangential orientation of the influent flow path.

Figure 4 is an elevational cross-sectional view of the mixing chamber shown in Figure 1 with a representation of a fluid vortex in the lower mixing chamber.

Figure 5 is an elevational perspective view of a second embodiment of a mixing chamber in accordance with the present invention.

Figure 6 is a cross-sectional view of an additional embodiment having two influent ports on the same horizontal plane with complementary influent flow paths.

Figure 7 is an elevational perspective view of an additional embodiment of the invention having rotatable stirring blades.

Figure 8 is an elevational perspective view of another preferred embodiment of this invention.

Figure 9 is an elevational cross sectional view along the lines 9-9 in Figure 8.

Figure 10 is an enlarged fragmentary view of the junction between the upper and lower chambers shown in Figure 9.

Figure 11 is an elevational perspective view of another preferred embodiment of this invention.

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third sterilizing filter is provided in the effluent stream from the second mixing chamber in an embodiment for use with a material which is to be sterilized.

5 In accordance with another aspect of the present invention, there is provided a method of reconstituting a powdered material in a buffer solution. In accordance with the method, a vortex mixing apparatus having a powdered culture media in a first mixing chamber therein is provided, the apparatus also having a buffer material in a second mixing  
10 chamber.

An influent fluid stream is introduced under pressure into the first mixing chamber for contacting the powdered culture media and creating a mixing vortex therein. Thereafter, the fluid stream is directed out of the first  
15 mixing chamber and into the second mixing chamber for contacting the buffer material.

In a preferred embodiment, the effluent stream from the second mixing chamber is directed through a sterilization filter and into a receiving bag. Preferably, the volume of  
20 the receiving bag, the volume of the powdered culture media and buffer are all coordinated so that the introduction into the first chamber of a sufficient volume of fluid to substantially fill the bag provides a unit volume of reconstituted culture media.

25 In accordance with a further aspect of the present invention, a parallel flow mixing apparatus is provided in which an incoming fluid stream is divided into two or more fluid streams, each of which in turn drives a separate mixing chamber. Variations of water-driven mixing include the water-  
30 driven vortex alone, or water-driven vortex together with an internal mixing blade. Alternatively, external water-driven mixing means may be used including an external water-driven turbine rotationally coupled with an internal mixing blade. Additional external mechanical mixing means, such as magnetic  
35 stir bar or rotationally coupled motor-driven external mixing means, are also provided.

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with the effluent stream from the sterilization filter. The connector is adapted to releasably engage a fluid receptacle for receiving the reconstituted product from the mixing apparatus.

5           In accordance with another aspect of the present invention, there is provided a method of reconstituting a powdered material in a buffer solution. In accordance with the method, a vortex mixing apparatus having a powdered culture media in a first mixing chamber therein is provided,  
10           the apparatus also having a buffer material in a second mixing chamber.

          An influent fluid stream is introduced under pressure into the first mixing chamber for contacting the powdered culture media and creating a mixing vortex therein.  
15           Thereafter, the fluid stream is directed out of the first mixing chamber and into the second mixing chamber for contacting the buffer material.

          In a preferred embodiment, the effluent stream from the second mixing chamber is directed through a sterilization  
20           filter and into a receptacle. Preferably, the volume of the receptacle bag, the volume of the powdered culture media and buffer are all coordinated so that the introduction into the first chamber of a sufficient volume of fluid to substantially fill the receptacle provides a unit volume of reconstituted  
25           culture media.

          In another embodiment a second mixing chamber is preferably provided in fluid communication with the effluent of the first mixing chamber, for containing a second concentrated material to be mixed with the incoming fluid  
30           stream. In a preferred embodiment, the first mixing chamber and second mixing chamber are in fluid communication with each other by way of a first filter. The filter is maintained within the fluid stream by a filter support structure on at least one, and preferably both sides of the filter.

35           The effluent stream from the second mixing chamber is provided with a second filter for substantially preventing the escape of undissolved materials therefrom, and, optimally, a

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laboratory there are a range of chemicals that are purchased as a powder or series of powders or as a series of concentrates and must be prepared prior to use. Other substances may be toxic so handling should be minimized. Some  
5 chemicals are required to be free of nucleases such as those found on human hands and require sterilization before use. Still others must be free from contaminants including dusts, bacteria, viruses and fungi. As a liquid these substances may have a predetermined shelf-life and while they may be  
10 inexpensive to purchase as a powder, they are considerably more expensive to purchase and receive in a prepackaged, filtered sterile liquid form.

#### Summary of the Invention

There is provided in accordance with one aspect of the  
15 present invention a mixing apparatus for mixing a concentrated material with an incoming fluid stream. The mixing apparatus comprises a housing having a substantially cylindrical mixing chamber therein for containing concentrated material to be mixed, and an influent port in the housing for providing fluid  
20 communication between the mixing chamber and a source of fluid. The influent port is aligned to direct incoming fluid along an axis which is generally tangential to the interior wall of the mixing chamber, thereby generating a rotational fluid velocity within the mixing chamber upon introduction of  
25 fluid under pressure. Preferably, a filter is provided in the effluent stream from the mixing chamber to substantially prevent the escape of unmixed powdered material from the mixing chamber.

A second mixing chamber is preferably provided in fluid  
30 communication with the effluent of the first mixing chamber, for containing a second concentrated material to be mixed with the incoming fluid stream. In a preferred embodiment, the first mixing chamber and second mixing chamber are in fluid communication with each other by way of a first filter. The  
35 effluent stream from the second mixing chamber is provided with a second filter which may be a sterilization filter. Preferably, a connector is provided in fluid communication

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then passed through a sterilizing filter. The media may thereafter be collected in a single large sterile vessel, or proportioned into several smaller sterile vessels.

5 Powdered tissue culture media has a very fine particle size and is hygroscopic. When mixed with water, it tends to "ball" or "clump." Thus, when reconstituting in water, sufficient agitation is required to break up any clumps that may form upon initial contact with water. For smaller batch sizes, sterile magnetic stir bars can be added to the  
10 dissolution container and the container is then placed on a magnetic stir plate. Additional manipulations are required to add stir bars to the dissolution containers. In a typical laboratory setting, magnetic stir plates are not a practical solution for large volume media preparation.

15 In addition, due to its hygroscopic nature, the media absorbs water when stored, especially in humid environments. Wet media has a shortened shelf-life, becomes lumpy and requires aggressive agitation to reconstitute. Thus, powdered media shelf life could be improved if it were provided in  
20 premeasured sealed and desiccated aliquots.

The reconstitution process requires several steps and several separate pieces of equipment. It generally requires at least one vessel, large enough to contain the entire final volume of reconstituted media, plus one or more vessels to  
25 receive the sterile media after filtration. The sterilized media is usually delivered into open top containers. Thus, most media preparation is done in a laminar flow hood. Processing large volumes of media in a hood is difficult because there is often not enough space to accommodate the  
30 containers and sterile media. A device that would permit the preparation of such a product with minimal physical contact and facilitate media preparation without the inconveniences described above would fulfill a long felt need in the scientific community.

35 There are a wide variety of solutions, the preparation of which requires the sequential dissolution or addition of components with minimum physical contact. In the research



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Cell culture media is available commercially either dry powder which is reconstituted by adding an appropriate volume of water, or as a pre-packaged liquid. There are also a number of additives that are typically added to the media before use. These include sodium bicarbonate, glutamine, additional buffers or antibiotics.

Pre-packaged liquid is sterile, aliquoted into convenient sizes and is ready to use. However, the media is typically light sensitive and has a prescribed shelf-life. Therefore, media must be ordered on a regular basis. It also should be stored under refrigeration and, in its prepackaged form, requires significant man-power time to unpackage and transport. Further, shipping costs of prepackaged liquid is becoming increasingly more expensive.

Powdered media is provided in bulk or in premeasured packages. It tends to have a longer shelf life, is less expensive and requires less storage space and handling time than the liquid form. However, the powdered media must be dissolved and aliquoted under sterile conditions. The increased handling and preparation time especially for large volume media preparation often makes pre-packaged liquid media the preferred choice despite the increased cost. Thus a powdered media that is easy to prepare, requires less storage space than liquid media and whose preparation requires minimal effort will be a significant improvement over the current art.

Reconstitution of powdered media is a several step process. To prepare a liquid media from a solid powder, a known amount of powder intended for a specific volume of media is measured out and added to a volume of distilled water which is typically slightly less than the final desired volume. The powder and water are stirred until the solid is completely dissolved. Then, a specific quantity of sodium bicarbonate is added and dissolved. Sodium bicarbonate and the powdered media must not be simultaneously added to the water, or a calcium carbonate precipitate forms. The pH may thereafter be adjusted using acid or base and additional water is added to increase the media to its final volume. The entire mixture is

DISSOLUTION APPARATUS  
Background of the Invention

5

10       The present invention relates to dissolution apparatus for dissolving an incoming fluid stream with a material to be mixed with the incoming fluid stream. More particularly, the present invention relates to dissolution apparatus specially adapted for reconstituting powdered cell culture media in predetermined unit volume amounts.

15       Viable animal cells and tissue in in vitro cultures have been known since the early 1900s. While animal cell culture today is a sophisticated technology, the basic culture technique has not changed since the beginning of the century. Cells or tissue, either primary or transformed, are grown in a liquid nutrient mixture generally referred to as "media."  
20       This media is a complex mixture of amino acids, vitamins, salts, and other components. It is often supplemented with 1-10% purified bovine fetal or newborn calf serum. Cell culture media and serum are available commercially from many sources.

25       While the basic cell culture technique has not changed appreciably over the years, the volume of cell culture and the accessibility of this laboratory technique has increased dramatically. Not only are more research laboratories, pharmaceutical and biotechnology companies employing tissue culture techniques but they are doing so, often, on a  
30       relatively large scale. A medical product related corporation may consume tens or hundreds of liters of liquid media a day and employ large numbers of laboratory technicians and scientists to generate antibodies, growth factors or purified protein from tissue culture for commercial use. Thus, between  
35       media supply costs and employee time there is a considerable expense associated with the tissue culture process today.